No Use is Disuse

Physical training in Duchenne muscular dystrophy

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1

General introduction

Duchenne muscular dystrophy (DMD) is the most common form of muscular dystrophy in childhood. Boys with DMD suffer from progressive loss of muscle strength and loss of functional abilities from a very young age. They lose the ability to ambulate in their early teenage years and the ability to lift their arms soon after the onset of wheelchair dependency. Because there is no cure for DMD, patient management is symptomatic directed at relieving complaints, supporting bodily functions and activities, and optimizing social participation and quality of life. Recommendations on physical training in boys with DMD are focused on avoiding disuse atrophy and other secondary complications of physical inactivity. However, these recommendations are based on limited evidence. This thesis describes the development and results of the No Use is Disuse (NUD) study, an assisted physical training program for boys with DMD that aimed to delay the loss of functional abilities secondary to disuse. Furthermore, this thesis describes the validation of three specific outcome measures (i.e., quantitative skeletal muscle ultrasound (QMUS) to assess the muscle histology, the Assisted 6-Minute Cycling Test (A6MCT) to assess physical endurance, and the Kidscreen-52 to assess Health Related Quality of Life (HRQoL)) for monitoring disease progression and the effects of interventions.

Duchenne muscular dystrophy

Pathophysiology

DMD affects approximately 1 in 5000 newborn boys.[1] Girls who inherit a DMD mutation usually do not develop the disease, because the pattern of inheritance is X-linked recessive. DMD is caused by mutations in the DMD gene, resulting in an absence, or near-absence (<3%), of the functional protein dystrophin.[2] Dystrophin forms a mechanical link between the actin filaments inside the muscle cells and the extracellular matrix, and it provides mechanical stability during muscle contractions (Figure 1). Absence of dystrophin results in fragile muscle fibers that are prone to contraction-induced injury, leading to ongoing cycles of degeneration and regeneration. These ongoing degenerative cycles cause chronic inflammation, impaired muscle tissue repair, and eventually a replacement of muscle fibers by fat and connective tissue.[3]

Clinical progression

The course of DMD distinguishes a presymptomatic stage, an early and late ambulatory stage, and an early and late non-ambulatory stage.[4] First, boys are in a presymptomatic stage in which they can still improve their functional abilities and do not show any balance or gait impairments.[5] Subsequently, they develop early symptoms including a waddling gait, an inability to run and jump properly, difficulty with climbing stairs and frequent falls.[4] Other early signs are pelvic girdle weakness indicated by the need for a Gowers' maneuver (i.e., taking support and pushing off with the hands on the thighs to be able to

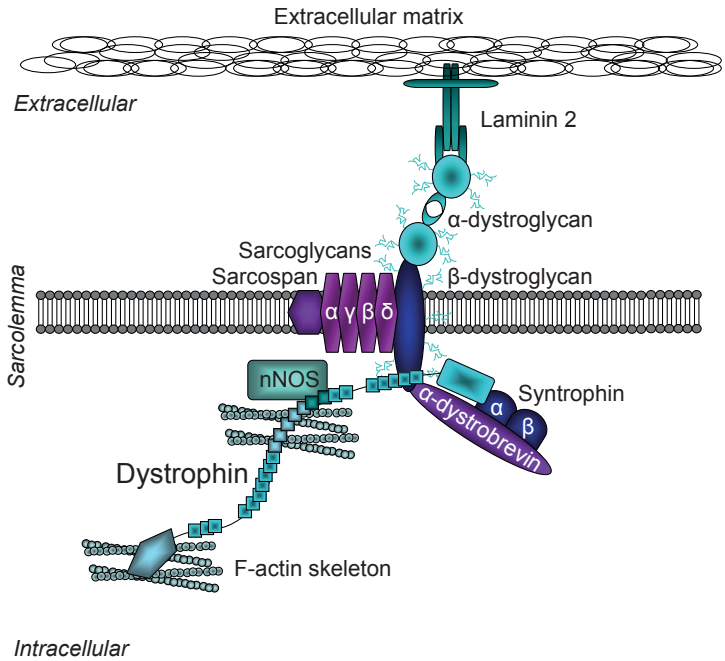

Figure 1 The location of dystrophin in skeletal muscle.

The dystrophin protein is located at the inside of the muscle cell membranes. It forms a mechanical link between the actin filaments of the cytoskeleton and, via its connexion to a group of other proteins in the cell membrane (i.e. the dystrophin-glycoprotein complex), the extracellular matrix. (Reprinted with permission from M. van Putten (2013). The influence of low dystropin levels on disease pathology in mouse models for Duchenne Muscular Dystrophy. Department of Human Genetics of the Leiden University Medical Center. Leiden, The Netherlands.)

extend the knees, hips and trunk when rising from the floor), and by pseudo hypertrophy of the calves.[6] The presence of these early signs often leads to a diagnosis around the age of 5 years.[7] After a plateau stage (early ambulatory stage), in which the functional abilities neither improve nor decline, walking becomes increasingly difficult in the late ambulatory stage and boys lose the ability to climb stairs and to rise from the floor.[5] Eventually, boys completely lose their walking ability and become, if untreated, wheelchair dependent at an average age of 9 to 10 years.[8-10] After the onset of wheelchair dependency, patients soon lose the ability to lift their arms against gravity (with only the distal muscles being able to overcome gravity) which results in severe limitations in motor capacities and in activities of daily living (ADL).[9,11,12] Without treatment, Duchenne adolescents or young adults die prematurely in their twenties or early thirties due to heart and/or respiratory failure.[13-15]

Treatment

Although promising therapies that aim to repair the disrupted dystrophin reading frame are now being developed[16], there is no cure for DMD yet and boys still have to rely on symptomatic treatment. Symptomatic treatment in DMD includes rehabilitative interventions such as the use of corticosteroids, the management of respiratory, cardiac and orthopedic complications, the psychosocial management of stress associated with the progressive character of the disease, the management of gastrointestinal, speech/swallowing and nutritional problems and physiotherapy directed at the maintenance of joint mobility.[4] Corticosteroids are the only proven treatment to slow down the loss of muscle strength and prolong the ability to walk.[8,17] Because there is no other cure, physiotherapy is important in the management of skeletal muscle complications such as contractures.[18]

Physiotherapy

The primary aim of physiotherapy in the management of DMD is to minimize the development of joint contractures and maintain muscle length and strength in order to maximize functional capacities at all stages of the disease.[19] The main techniques by which this aim is pursued are muscle stretching, joint splinting and physical training, but no clear guidelines on any of these methods exist. As a consequence, the applied techniques vary greatly. Currently, physical training often receives little attention in the management of DMD, since many physiotherapists focus on passive stretching.[20] The reason for this is that they do not want to overburden the boys with too much training as exhaustion can be detrimental to the muscles, which can negatively affect training compliance. However, physiotherapists should be able to develop a safe training program for Duchenne patients that is relevant to their ADL and compatible with their actual disease status.[18-20] Most importantly, physiotherapists need to be able to help these boys to remain as active as possible within their individual possibilities to prevent further physical deterioration due to disuse. But what exactly does the term 'disuse' stand for in the literature?

Disuse

Physical inactivity should be avoided in boys with DMD, because unloading the muscles results in a secondary physical deterioration.[20,21] To illustrate the negative effects of physical inactivity, both bed rest for a simple fever and immobility after a lower limb fracture can lead to permanent loss of walking ability.[22-25] Nevertheless, boys with DMD are particularly at risk of a sedentary lifestyle.[26] This is not only because of the ongoing muscle weakness, but also because of the progressively limited physical and social activities that they are able to engage in.[26] For example, the increasing amount of energy needed to ambulate and the increasing frequency of falls (without being able to stand up independently) will make boys move less and 'force' them to rely on a wheelchair. A wheelchair in its turn forces the boys to function within its confines, which accelerates the loss of reaching and lifting power of the arms. Thus, it is likely that the physical

deterioration in DMD is partly caused by 'disuse', which is defined here as a discrepancy between individual (latent) capacity and actual performance in daily life, as a result of a sedentary life style.[21] From this perspective, the well-known saying "use it or lose it" seems to be highly applicable to boys with DMD. Physical training might therefore be beneficial to these patients if it prevents disuse and the related deterioration of the musculoskeletal and cardiovascular system.

Physical training for Duchenne muscular dystrophy

Available scientific evidence

No clear training prescriptions exist for physical training in boys with DMD and only little research in this area has been conducted. Animal studies with mice who suffer from X-linked muscular dystrophy (mdx mice, the most commonly used mouse model in DMD research) showed that dynamic voluntary exercises (such as wheel running) had significant positive effects on muscle strength and fatigue resistance[27,28], but they also showed that eccentric exercises accelerated muscle fiber injuries.[29] Applying the results from animal studies to boys with DMD is, however, difficult. The research on training in DMD in man is limited to three studies investigating the effect of resistance exercises in ambulant boys.[30-32] These studies concluded that submaximal resistance exercises did not cause any harm, but had only limited positive effects on muscle strength and timed functional tests (such as the time it took to walk 23 feet). A limitation of these studies was that they were conducted in uncontrolled settings, i.e. all boys received the training without comparison with a control group or control condition. No randomized controlled trial (RCT) concerning training has as yet been performed in boys with DMD. In general, the number of RCTs on physical training in muscle diseases is limited to five trials that included adults. These trials showed that moderate-intensity strength training and/or aerobic exercise training had limited value in patients with myotonic dystrophy type I, facioscapulohumeral muscular dystrophy, dermatomyositis and poliomyositis, but did not cause any harm either. A combination of strength training and aerobic exercises has been shown to increase submaximal endurance capacity in patients with mitochondrial myopathy. However, based on these trials no training advice can be deduced for boys with DMD.[33]

Further intervention studies on physical training

The lack of RCTs on physical training in boys with DMD underscores the need for well controlled intervention studies in this area. Based on the current knowledge from animal studies and on pathophysiology, some recommendations can be made for such interventions. Boys with DMD are recommended to regularly participate in mildly intensive functional activities, including swimming and recreation-based exercises in the community, to avoid disuse.[20] It is advised that eccentric (lengthening) exercises and exhausting high-load resistance

exercises are avoided.[20,34-36] During eccentric exercises, actin and myosin filaments are pulled apart and cause damage to the stretched sarcomeres. This results in a loss of force-generating capacity and, with an inability to quickly repair this damage, subsequent fiber degeneration. In particular muscles with a lack of dystrophin, such as in DMD, are vulnerable to loss of force-generating capacity and fiber generation, probably because of the role that dystrophin plays in transmitting forces from the intracellular cytoskeleton to the extracellular matrix.[35] Assisted physical training in which dynamic support is provided by a mechanic or electric device may be a feasible and a safe alternative for resistance training. Assistance would allow boys with severe muscle weakness to train their extremities without becoming exhausted, e.g. mechanical assistance by means of a powered or unpowered device reduces the muscle strength needed to train while subjects still contract their muscles. Yet, controlled research is needed to increase insight into what type of physical training boys with DMD can safely and effectively participate in throughout the course of their disease. This should lead to the development of more precise and evidence-based physical training recommendations reducing the uncertainty in boys with DMD and their families. In addition, appropriate outcome measures are needed to assess the effects of such physical training programs.

Outcome measures for intervention studies in Duchenne muscular dystrophy

Prerequisites

Valid and reliable outcome measures are needed to evaluate the effect of training programs in DMD. Such measures should be sufficiently responsive to measure changes over time and should be related to clinical milestones such as the loss of the ability to walk.[37] Furthermore, outcome measures should assess the varying levels of functional abilities and activities that are observed at the different ages.[38] This means that they should be feasible for ambulant as well as wheelchair-dependent boys with DMD.

Current set of clinical outcome measures

An overview of the most frequently used outcome measures in clinical trials in DMD is shown in Table 1. The most commonly used primary outcome measure in clinical trials is the Six-Minute Walk Test (6MWT), a submaximal physical endurance test.[39] Although physical endurance tests are indeed clinically relevant since they are related to motor functioning in daily life[39,40], a disadvantage of the 6MWT is that this test is not feasible for wheelchair-dependent boys. A cycling test could be an alternative for more severely impaired boys, but the load of regular bicycle ergometers is often too high for these boys.[41,42]

Other proposed clinical outcome measures for boys with DMD include assessments of muscle strength, joint range of motion, activities of daily living, timed (functional) tests,

Table 1 Overview of frequently used outcome measures in DMD

Construct	Instrument	Advantages	Disadvantages
Muscle histology	Biopsy	Accurate	Invasive Sedation needed
	MRI	All muscles can be visualized	Expensive Sedation may be needed
	QMUS	Fast and child-friendly	Normal values for each device required
Muscle strength	HHD	Continuous data Distinguishes between MRC 4 and 5	Not sensitive for muscles with strength MRC <3
	MRC scale	Fast No HHD device needed	Not sensitive for muscles with strength MRC ≥4
Joint range of motion	Goniometry	Fast and practical	Test-retest reliability limited
	3D motion analysis	Accurate and extensive	Expensive Time consuming Often not available
Physical endurance	6MWT	No specific equipment needed Clear instructions Meaningful to patients	Not appropriate for wheelchair-dependent boys Fall risk
Motor function	Timed tests	Fast No specific equipment needed	Difficult to analyze if a patient loses the ability to perform a test
	HFMS/M-HFMS	Minimal equipment needed	Not appropriate for wheelchair-dependent boys Ceiling effect
	EK	Meaningful to the patients	Not appropriate for ambulant boys Sensitiveness is limited
	MFM	Can be used in both ambulant and wheelchair-dependent boys	Generic Limited number of items on proximal arm functions
	NSAA	Responsive to changes in motor functions over time	No items on arm function Not appropriate for wheelchair-dependent boys
	Jebsen Test	Continuous data	Assesses primarily hand-function Test kit needed
HRQoL	PedsQL DMD	NMD module available	No questions on psychological well-being

MRI, Magnetic Resonance Imaging; QMUS, Quantitative Muscle Ultrasound; HHD, Hand-Held Dynamometry; MRC, Medical Research Council; 3D three dimensional; 6MWT, Six-Minute Walk Test; HFMS, Hammersmith Functional Motor Scale; M-HFMS, Modifief-Hammersmith Functional Motor Scale; EK, Egen Klassification; MFM, Motor Function Measure; NSAA, North Star Ambulatory Assessment; Jebsen Test, Jebsen-Taylor Hand Function Test; PedsQL DMD, Pediatric Quality of Life Inventory Duchenne Muscular Dystrophy Module
(Table based on Mercuri et al. (2008), and adjusted.)

and scales of general motor functioning.[4,37,43,44] Especially the latter type of scale provides a composite score of various motor functions that allows to monitor disease progression and possible responsiveness to treatment. The Motor Function Measure (MFM) is the only scale of this type that assesses the functional abilities of both the lower and upper limbs. As such, it can be applied to ambulant and non-ambulant patients with a neuromuscular disorder (NMD).[45]

Quantitative muscle imaging

Quantitative muscle imaging is a relevant child-friendly supplement to the current set of clinical outcome measures in boys with DMD. It quantifies structural muscle changes underlying the functional deterioration.[46,47] It is a non-invasive technique that is not influenced by fatigue, motivation or the ability to cooperate. To achieve motivation and cooperation is a challenge inherent in testing children, but especially in boys with DMD since cognitive deficits are present in approximately one-third of the patients.[48,49] In particular the autism spectrum disorder and attention-deficit hyperactivity disorder (ADHD), affecting 3.1% and 11.7% of the boys with DMD respectively, can cause difficulty with following directions needed to perform clinical tests.[50] Magnetic resonance imaging (MRI) is a highly reliable quantitative muscle imaging technique that assesses muscle histology in boys with DMD[46,51], but it is expensive and not always available, while sedation is often needed in children. In contrast to MRI, quantitative muscle ultrasound (QMUS) is cheap, fast, child-friendly and does not require sedation. QMUS quantifies fatty infiltration and intramuscular fibrosis and distinguishes children with and without DMD with a positive predictive value of 86% and a negative predictive value of 95%.[52,53,47] Yet, it is unknown whether QMUS is also sensitive to measure progressive changes in muscle structure over time and how these changes might relate to clinical disease progression and loss of functional abilities.

Health related quality of life

Finally, there is a need to determine how boys with DMD cope with situations in everyday life[26] and how they perceive their own health status. Therefore, authorities such as the international Food and Drug Administration (FDA) have recognized that the inclusion of patient-reported outcome measures (PROM), such as Health Related Quality of Life (HRQoL) questionnaires, are useful in clinical trials.[54] HRQoL refers to an individual's perception of health and of one's position in life. Although it is well-known that boys with DMD report lower physical well-being compared to healthy boys, little is known about the relationship between their HRQoL and disease severity as assessed with existing outcome measures.[43]

Aim of this thesis

The main aim of the research presented in this thesis was to increase insight into the feasibility, safety and effectiveness of assisted physical training in boys with DMD who are ambulatory or wheelchair-dependent. In order to evaluate the effectiveness of the assisted physical training, three specific outcome measures were developed and/or validated. These aims led to the following main research questions that are addressed in this thesis:

1) Are QMUS, the Assisted Six-Minute Cycling Test (A6MCT) and HRQoL valid and/or responsive outcome measures to evaluate the effects of a physical training intervention in boys with DMD?
2) Is assisted physical training feasible, safe and effective to delay the functional deterioration in ambulatory and wheelchair-dependent children with DMD?

It was hypothesized that assisted physical training would delay the secondary functional deterioration due to disuse in DMD and that this delay could be assessed with QMUS, the A6MCT and HRQoL.

Outline of this thesis

This thesis is divided into two parts addressing the previously specified main research questions. Part one encompasses the studies on outcome measures, whereas part two addresses the effectiveness of two assisted physical training programs.

Part 1 Outcome measures

Chapter 2 starts with the investigation of the validity and responsiveness of QMUS by means of a longitudinal follow-up of 18 boys with DMD. In **chapter 3**, the development of the assisted 6-minute cycling test (A6MCT) meant to assess physical endurance is described. Its validity was examined in both healthy boys and boys with DMD. **Chapter 4** describes the HRQoL of boys with DMD who are either ambulant or wheelchair-dependent as well as the HRQoL perception of the boys' parents as assessed with the KIDSCREEN-52 questionnaire.

Part 2 Physical training

Chapter 5 describes the protocol of the No Use is Disuse (NUD) study. The NUD study is the first study in boys with DMD that examines whether assisted physical training is safe and beneficial to preserve muscle endurance and functional abilities. The study consists of two assisted physical training programs: study 1 "Dynamic leg and arm training for ambulant and recently wheelchair-dependent boys with DMD" (an RCT), and study 2 "Functional training with arm-support for boys with DMD who have been confined to a wheelchair for several years" (a feasibility study). The results of these two physical training programs are described in **chapter 6** and **chapter 7**, respectively.

In **chapter 8**, the methods and results of the studies in this thesis are summarized and subsequently discussed. Furthermore, recommendations for further research are provided.

References

1. Mendell JR, Lloyd-Puryear M. Report of MDA muscle disease symposium on newborn screening for Duchenne muscular dystrophy. Muscle Nerve 2013;48:21-26.
2. Hoffman EP, Brown RH, Jr., Kunkel LM. Dystrophin: the protein product of the Duchenne muscular dystrophy locus. Cell 1987;51:919-928.
3. Deconinck N, Dan B. Pathophysiology of duchenne muscular dystrophy: current hypotheses. Pediatric neurology 2007;36:1-7.
4. Bushby K, Finkel R, Birnkrant DJ et al. Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and pharmacological and psychosocial management. Lancet Neurol 2010;9:77-93.
5. Mazzone E, Vasco G, Sormani MP et al. Functional changes in Duchenne muscular dystrophy: a 12-month longitudinal cohort study. Neurology 2011;77:250-256.
6. Cros D, Harnden P, Pellissier JF, Serratrice G. Muscle hypertrophy in Duchenne muscular dystrophy. A pathological and morphometric study. Journal of neurology 1989;236:43-47.
7. Ciafaloni E, Fox DJ, Pandya S et al. Delayed diagnosis in duchenne muscular dystrophy: data from the Muscular Dystrophy Surveillance, Tracking, and Research Network (MD STARnet). The Journal of pediatrics 2009;155:380-385.
8. Biggar WD, Harris VA, Eliasoph L, Alman B. Long-term benefits of deflazacort treatment for boys with Duchenne muscular dystrophy in their second decade. Neuromuscul Disord 2006;16:249-255.
9. McDonald CM, Abresch RT, Carter GT et al. Profiles of neuromuscular diseases. Duchenne muscular dystrophy. Am J Phys Med Rehabil 1995;74:S70-S92.
10. Vuillerot C, Girardot F, Payan C et al. Monitoring changes and predicting loss of ambulation in Duchenne muscular dystrophy with the Motor Function Measure. Dev Med Child Neurol 2010;52:60-65.
11. Bartels B, Pangalila RF, Bergen MP et al. Upper limb function in adults with Duchenne muscular dystrophy. Journal of rehabilitation medicine : official journal of the UEMS European Board of Physical and Rehabilitation Medicine 2011;43:770-775.
12. Uchikawa K, Liu M, Hanayama K, Tsuji T, Fujiwara T, Chino N. Functional status and muscle strength in people with Duchenne muscular dystrophy living in the community. Journal of rehabilitation medicine : official journal of the UEMS European Board of Physical and Rehabilitation Medicine 2004;36:124-129.
13. Bach JR, Martinez D. Duchenne muscular dystrophy: continuous noninvasive ventilatory support prolongs survival. Respir Care 2011;56:744-750.
14. Eagle M, Bourke J, Bullock R et al. Managing Duchenne muscular dystrophy--the additive effect of spinal surgery and home nocturnal ventilation in improving survival. Neuromuscul Disord 2007;17:470-475.
15. Kohler M, Clarenbach CF, Bahler C, Brack T, Russi EW, Bloch KE. Disability and survival in Duchenne muscular dystrophy. Journal of neurology, neurosurgery, and psychiatry 2009;80:320-325.
16. Goemans NM, Tulinius M, van den Akker JT et al. Systemic administration of PRO051 in Duchenne's muscular dystrophy. N Engl J Med 2011;364:1513-1522.
17. Manzur AY, Kuntzer T, Pike M, Swan A. Glucocorticoid corticosteroids for Duchenne muscular dystrophy. Cochrane database of systematic reviews 2008(1):CD003725.
18. Lovering RM, Porter NC, Bloch RJ. The muscular dystrophies: from genes to therapies. Physical therapy 2005;85:1372-1388.
19. Eagle M. Report on the muscular dystrophy campaign workshop: exercise in neuromuscular diseases Newcastle, January 2002. Neuromuscul Disord 2002;12:975-983.
20. Bushby K, Finkel R, Birnkrant DJ. Diagnosis and management of Duchenne muscular dystrophy, part 2: implementation of multidisciplinary care. Lancet Neurol 2010;9:177-189.
21. McDonald CM. Physical activity, health impairments, and disability in neuromuscular disease. Am J Phys Med Rehabil 2002;81:S108-S120.
22. McDonald DG, Kinali M, Gallagher AC et al. Fracture prevalence in Duchenne muscular dystrophy. Dev Med Child Neurol 2002;44:695-698.
23. Vestergaard P, Glerup H, Steffensen BF. Fracture risk in patients with muscular dystrophy and spinal muscular atrophy. J Rehabil Med 2001;33:150-155.

24. Bar-Or O, Rowland TW. Neuromuscular and musculoskeletal diseseases. In: Bar-Or O, Rowland TW, editors. Pediatric exercise medicine. From physiologic principles to health care application. United States of America: Human Kinetics; 2004. p 269-321.
25. Vignos PJ, Jr. Physical models of rehabilitation in neuromuscular disease. Muscle Nerve 1983;6:323-338.
26. Bendixen RM, Senesac C, Lott DJ, Vandenborne K. Participation and quality of life in children with Duchenne muscular dystrophy using the International Classification of Functioning, Disability, and Health. Health and quality of life outcomes 2012;10:43.
27. Hayes A, Williams DA. Beneficial effects of voluntary wheel running on the properties of dystrophic mouse muscle. J Appl Physiol 1996;80:670-679.
28. Call JA, McKeehen JN, Novotny SA, Lowe DA. Progressive resistance voluntary wheel running in the mdx mouse. Muscle Nerve 2010;42:871-880.
29. Vilquin JT, Brussee V, Asselin I, Kinoshita I, Gingras M, Tremblay JP. Evidence of mdx mouse skeletal muscle fragility in vivo by eccentric running exercise. Muscle Nerve 1998;21:567-576.
30. Scott OM, Hyde SA, Goddard C, Jones R, Dubowitz V. Effect of exercise in Duchenne muscular dystrophy. Physiotherapy 1981;67:174-176.
31. de Lateur BJ, Giaconi RM. Effect on maximal strength of submaximal exercise in Duchenne muscular dystrophy. Am J Phys Med 1979;58:26-36.
32. Vignos PJ, Jr., Watkins MP. The effect of exercise in muscular dystrophy. JAMA 1966;1971:843-848.
33. Voet NB, van der Kooi EL, Riphagen II, Lindeman E, van Engelen BG, Geurts AC. Strength training and aerobic exercise training for muscle disease. Cochrane database of systematic reviews 2010(1):CD003907.
34. Carter GT, Abresch RT, Fowler WM, Jr. Adaptations to exercise training and contraction-induced muscle injury in animal models of muscular dystrophy. Am J Phys Med Rehabil 2002;81:S151-161.
35. Allen DG. Eccentric muscle damage: mechanisms of early reduction of force. Acta physiologica Scandinavica 2001;171:311-319.
36. Petrof BJ. The molecular basis of activity-induced muscle injury in Duchenne muscular dystrophy. Molecular and cellular biochemistry 1998;179:111-123.
37. Mercuri E, Mayhew A, Muntoni F. Towards harmonisation of outcome measures for DMD and SMA within TREAT-NMD; report of three expert workshops: TREAT-NMD/ENMC workshop on outcome measures, 12th--13th May 2007, Naarden, The Netherlands; TREAT-NMD workshop on outcome measures in experimental trials for DMD, 30th June--1st July 2007, Naarden, The Netherlands; conjoint Institute of Myology TREAT-NMD meeting on physical activity monitoring in neuromuscular disorders, 11th July 2007, Paris, France. Neuromuscul Disord 2008;18:894-903.
38. Bushby K, Connor E. Clinical outcome measures for trials in Duchenne muscular dystrophy: report from International Working Group meetings. Clinical investigation 2011;1:1217-1235.
39. Laboratories ATSCoPSfCPF. ATS statement: guidelines for the six-minute walk test. American journal of respiratory and critical care medicine 2002;166:111-117.
40. Mazzone E, Martinelli D, Berardinelli A et al. North Star Ambulatory Assessment, 6-minute walk test and timed items in ambulant boys with Duchenne muscular dystrophy. Neuromuscul Disord 2010;20:712-716.
41. Bar-Or O, Rowland TW. Procedure for exercise testing in children. In: Bar-Or O, Rowland TW, editors. Pediatric exercise medicine. From physiologic principles to health care application. United States of America: Human Kinetics; 2004. p 343-365.
42. Tirosh E, Bar-Or O, Rosenbaum P. New muscle power test in neuromuscular disease. Feasibility and reliability. Am J Dis Child 1990;144:1083-1087.
43. Mayhew JE, Florence JM, Mayhew TP et al. Reliable surrogate outcome measures in multicenter clinical trials of Duchenne muscular dystrophy. Muscle Nerve 2007;35:36-42.
44. Brooke MH, Griggs RC, Mendell JR, Fenichel GM, Shumate JB, Pellegrino RJ. Clinical trial in Duchenne dystrophy. I. The design of the protocol. Muscle Nerve 1981;4:186-197.
45. Berard C, Payan C, Hodgkinson I, Fermanian J. A motor function measure for neuromuscular diseases. Construction and validation study. Neuromuscul Disord 2005;15:463-470.
46. Kinali M, Arechavala-Gomeza V, Cirak S et al. Muscle histology vs MRI in Duchenne muscular dystrophy. Neurology 2011;76:346-353.

47. Pillen S, Tak RO, Zwarts MJet al. Skeletal muscle ultrasound: correlation between fibrous tissue and echo intensity. Ultrasound in medicine & biology 2009;35(3):443-446.
48. Cotton SM, Voudouris NJ, Greenwood KM. Association between intellectual functioning and age in children and young adults with Duchenne muscular dystrophy: further results from a meta-analysis. Dev Med Child Neurol 2005;47:257-265.
49. Darke J, Bushby K, Le Couteur A, McConachie H. Survey of behaviour problems in children with neuromuscular diseases. European journal of paediatric neurology : EJPN : official journal of the European Paediatric Neurology Society 2006;10:129-134.
50. Hendriksen JG, Vles JS. Neuropsychiatric disorders in males with duchenne muscular dystrophy: frequency rate of attention-deficit hyperactivity disorder (ADHD), autism spectrum disorder, and obsessive--compulsive disorder. Journal of child neurology 2008;23:477-481.
51. Forbes SC, Walter GA, Rooney WD et al. Skeletal Muscles of Ambulant Children with Duchenne Muscular Dystrophy: Validation of Multicenter Study of Evaluation with MR Imaging and MR Spectroscopy. Radiology 2013;269:198-207.
52. Pillen S, Verrips A, van Alfen N, Arts IM, Sie LT, Zwarts MJ. Quantitative skeletal muscle ultrasound: diagnostic value in childhood neuromuscular disease. Neuromuscul Disord 2007;17:509-516.
53. Reimers K, Reimers CD, Wagner S, Paetzke I, Pongratz DE. Skeletal muscle sonography: a correlative study of echogenicity and morphology. Journal of ultrasound in medicine : official journal of the American Institute of Ultrasound in Medicine 1993;12:73-77.
54. Varma R, Richman EA, Ferris FL, 3rd, Bressler NM. Use of patient-reported outcomes in medical product development: a report from the 2009 NEI/FDA Clinical Trial Endpoints Symposium. Investigative ophthalmology & visual science 2010;51:6095-6103.

Part 1

Outcome measures for Duchenne muscular dystrophy

2

Quantitative muscle ultrasound is a promising longitudinal follow-up tool in Duchenne muscular dystrophy

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Abstract

Responsive outcome measures are needed to follow the disease status of Duchenne muscular dystrophy (DMD) patients, as new therapeutic approaches become available for affected boys. Quantitative muscle ultrasound (QMUS) is potentially an attractive follow up tool for DMD because it reflects the severity of the dystrophic process without the need for invasive procedures, by quantifying echo intensity (i.e. mean grey level of muscle images) and muscle thickness. We performed a longitudinal follow-up of lower and upper extremity QMUS in 18 DMD patients and compared this with physical functioning in 11 of these patients. QMUS could be performed in every patient, and no patient was subjected to more than a total of 20 min of ultrasound scanning time for this study. As expected we found a significant increase of echo intensity with age, reflecting increasing dystrophic muscle changes. This increase was related to ambulatory status, functional grading, muscle strength and motor ability. Our study establishes QMUS as a practical and child-friendly tool for the longitudinal follow up of DMD patients.

Introduction

Duchenne muscular dystrophy (DMD) is the most common debilitating neuromuscular disorder affecting young boys and their families. In the past decade new and promising therapeutic approaches have become available that need validation in practice. For this, responsive outcome measures are needed to quantify the disease status of boys with DMD. Such outcome measures also need to be practical, feasible in young children and patient friendly, and should therefore preferably be noninvasive.

The frequently used battery of clinical outcomes for DMD consists of quantitative muscle strength tests, the Six-Minute Walk Test[1], functional scales and timed function tests.[2] Although these outcome measures are clinically meaningful and have an immediate value to the boys and their parents, they also have their limitations. For example, sensitivity is often limited and ceiling effects exist for functional scales such as the Hammersmith Scale for Assessment of Motor Ability.[2,3] Likewise, muscle strength tests have limited correlation with functionality because a small reduction in strength is often accompanied by a large reduction in functional ability when a paresis is already present.[4] Specific testing can be difficult in the younger children for whom new therapies will preferably be intended. Taken together with the increased risk for cognitive[5] and neurobehavioral problems[6] in DMD, measuring these boys' physical and functional capacities can be challenging.

Quantitative muscle ultrasound (QMUS) is a child-friendly imaging technique that is fast, painless and does not require sedation or anaesthesia.[7,8] It provides an accurate and reliable tool to distinguish children with and without a neuromuscular disorder with high predictive values and can visualize the intramuscular fibrosis and fatty infiltration in DMD as a homogeneous increase of muscle echo intensity (i.e. the muscle becomes more echogenic, so that its image appears more uniformly filled with grey speckles).[7,9-12]

Quantitative muscle imaging could be a relevant supplement to the current battery of clinical outcome measures, as it objectifies structural muscle changes without being influenced by fatigue, verbal understanding or cooperation, and can thus potentially quantify the pathology underlying deterioration of physical functioning without the need for muscle biopsy or sedated MR imaging. A recent cross-sectional study among 39 DMD patients has shown that the quantitatively determined muscle echo intensity of the elbow flexors linearly increased with age and correlated with physical functioning and disease severity.[13] Using grayscale analysis, the echo intensity in healthy children does not change with age until they reach adulthood[8,12], although the use of another quantifying technique showed a possible increase in calibrated muscle backscatter in a plot of the elbow flexors over time.[13] Muscle echo intensity does increase significantly with age in healthy adults, probably as a result of age-related muscle replacement by fat and fibrous tissue.[13-16]

It is suspected but not yet investigated that echo intensity and muscle thickness of lower extremity muscles correlate with age and disease severity in DMD boys. It is also still unknown whether quantitatively assessed echo intensity and muscle thickness are sensitive enough to measure progressive changes in muscle structure over time or how strongly these changes are associated with clinical disease progression and loss of function. The aim of our study was therefore to perform a longitudinal follow up of the changes and relation of lower and upper extremity QMUS with age, disease severity and physical functioning in a cohort of DMD boys. The objective was to assess whether QMUS can be used as a responsive follow up tool in upcoming treatment trials for DMD.

Methods

Design and patients

The longitudinal observational cohort study was conducted at the Radboud University Nijmegen Medical Centre. Nineteen boys with a DNA-confirmed diagnosis of DMD who regularly visited the out-patient clinic of the Radboud University Nijmegen Medical Centre were included. Data were obtained in a period without changes in the boys' glucocorticoids regimen. Follow-up data were obtained from November 2006 to April 2010. Time intervals between measurements were allowed to vary. As the clinical and ultrasound assessments were part of our routine multidisciplinary care for DMD, no specific informed consent was obtained, but our institutional review board approved non-invasive skeletal muscle ultrasound data collection for this group of patients. Data were handled according to the guidelines for Good Clinical Practice.

Clinical assessments

Clinical assessments included grading of the ambulatory status (ambulant versus wheelchair-dependent), functional grading scales for the lower and upper extremity (Vignos and Brooke scales; see below), quantitative muscle testing of elbow flexors and knee extensors using dynamometry, and assessments of disease severity using the Childhood Myositis Assessment Scale and the Hammersmith Motor Ability Scale (see below). Unfortunately, our routine care did not yet include the Six-Minute Walk Test for endurance. Measurements were performed by three experienced pediatric physiotherapists. All clinical assessments were performed within 3 months of the QMUS measurements.

Functional grading

The Vignos functional grade, as described by Brooke in 1981[17], grades the severity of the involvement of the hips and legs on a scale from 1 ("Walks and climbs without assistance") to 10 ("Is confined to bed"). The Brooke functional grade grades the severity of the involvement of the arms and shoulders on a scale from 1 ("Starting with the arms at the

sides, the patient can abduct the arms in a full circle until they touch above the head") to 7 ("Cannot raise hands to mouth and has no useful function of hands").[17] Both functional grades have been shown to have good inter- and intra-rater reliability[17,18] and to correlate moderately with muscle strength[19] in boys with DMD.

Quantitative muscle strength testing

Muscle strength of the elbow flexors (left side) and knee extensors (right side) was assessed with a hand-held dynamometer (MicroFet II, Biometrics, Almere, The Netherlands). Patient and therapist position, stabilization, myometer position and instructions were standardized[20]. Measurements were performed using the "break technique".[20] Both muscle groups were tested three times at 30 s intervals and the highest value of these contractions was recorded. Measurement results were recorded in Newton. Z-scores were calculated from normal values (obtained by subtracting the normal value from the measured value and then dividing the difference by the SD of the normal value) established by Beenakker et al. (2001).[20] All results are presented as z-scores. Previous studies have shown good inter-rater reliability[21] and moderate correlations of dynamometry with functional timed tests[4] in boys with DMD.

Childhood Myositis Assessment Scale

As the Childhood Myositis Assessment Scale (CMAS) is used routinely as a follow-up tool for children with neuromuscular disorders at our centre, we assessed the severity of muscle involvement with this quantitative, observational, performance-based outcome measure which was originally developed for children with juvenile idiopathic inflammatory myopathies (IIM).[22] The CMAS consists of 14 physical tests which mainly assess proximal and axial muscle groups. The maximum score is 52 points with a higher score indicating a greater muscle function, strength and endurance. Previous studies have shown good inter- and intra-rater reliability, construct validity and responsiveness in children with juvenile IIM.[22,23] No studies have yet been conducted to assess the feasibility of the CMAS in boys with DMD.

Hammersmith Motor Ability Scale

Motor ability was assessed using the Hammersmith Motor Ability Scale (HMAS). The HMAS consists of 20 assessments of axial and lower extremity abilities. Each item is scored on a 3-point scale (2 = succeeds, 1 = succeeds with minimal assistance, 0 = unable to perform) and the scale has a maximum score of 40. A previous study has shown a high inter-rater reliability and a strong correlation with a total muscle strength score in boys with DMD.[3]

Quantitative muscle ultrasound measurements

Assessments were conducted with a real-time ultrasound scanner (IU22, Philips Healthcare, Eindhoven, The Netherlands) and a 17-5 MHz broadband linear transducer. Machine

settings were determined experimentally to achieve muscle ultrasound images in which healthy muscle and subcutaneous tissue appeared relatively black with strong reflections of fascia and bone. System settings were kept constant throughout the study, using a gain of 70, compression of 55, the time gain compensation switches in neutral position and the image focal zone at a depth of 1.0-2.5 cm. Power output was set so that the Tissue Index was 0.1 and the Mechanical Index was 0.8. We chose Gray Map #2 as this was the most linear look-up table, and we set 2D image optimization to "Gen" and persistency to "Low". Additional image enhancement settings (e.g. Sono-CT and Xres) were not applied.

Boys were examined supine with their arms and legs extended (as much as possible in case of contractures) and their muscles relaxed. Quantitative ultrasound measurements were performed according to a standard protocol as previously described.[8] A generous amount of contact gel was used to minimize the required pressure of the transducer on the skin. All scans were made in the transverse plane with a standard transducer location corresponding to the muscle belly, at the following anatomical sites: the biceps brachii muscle (BB) at two-thirds of the distance from the acromion to the antecubital crease; the forearm flexors (FF) at two-fifths of the distance from the antecubital crease to the distal end of the radius; the rectus femoris muscle (RF) halfway along the line from the anterior superior iliac spine to the superior aspect of the patella; and the tibialis anterior muscle (TA) at one-quarter of the distance from the inferior aspect of the patella to the lateral malleolus.

For each muscle three consecutive measurements were made to minimize variation in echo intensity during analysis and the screen images of these measurements were stored offline in DICOM format. We did not use zoom or change the depth settings during the study as this would potentially alter echo intensity. As oblique scanning angles can lead to incorrect muscle thickness and echo intensity measurements, this was avoided by adjusting the angle of the probe until the best bone echo was obtained in every image.

The captured images were analyzed offline for echo intensity by means of computer-assisted grayscale histogram analysis, using custom software (Qumia) developed at our center. In each of the stored muscle images, a region of interest (ROI) was manually selected. The ROI was defined as the region that included as much muscle mass as possible without bone and fascia. Next, the software automatically selected the upper one-third of each ROI from the depth of the entire cross sectional area measured from the most superficial pixel to the deepest pixel within the ROI (Figure 1). This was done because of attenuation of the ultrasound beam in more severely affected dystrophic muscles (Figure 2), which would falsely lower the mean muscle echo intensity.[12] From the selected region the mean muscle echo intensity was calculated based on a histogram analysis.

As this study was performed with a different ultrasound device than that used to establish our database of normal values[8], we converted all echo intensity values using a meat phantom and the conversion equation previously established and described.[24] This conversion resulted in reliable results.[24]

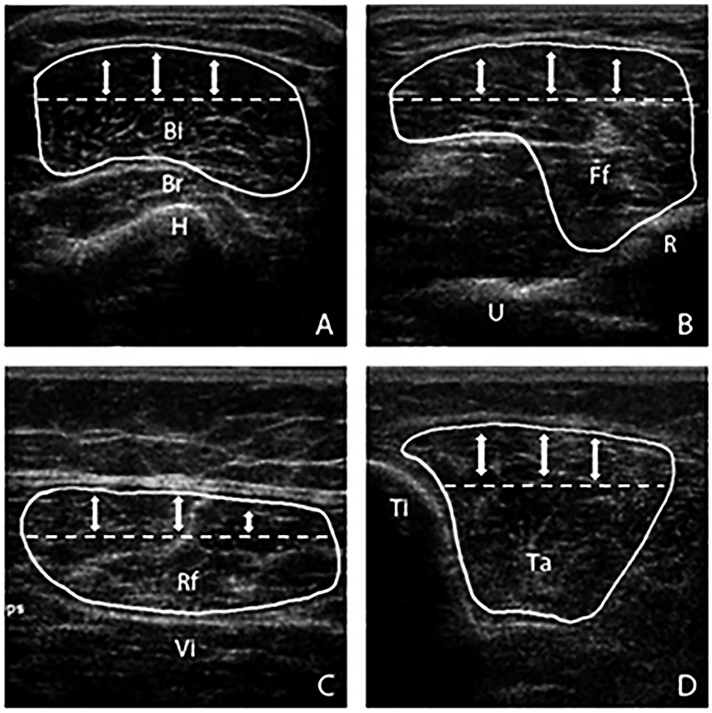

Figure 1 Quantitative ultrasound measurements were performed for the biceps brachii muscle (**A**), forearmflexors (**B**), rectus femoris muscle (**C**) and tibialis anterior muscle (**D**).

A region of interest was selected for each muscle and included as much muscle mass as possible without bone and fascia (white lasso). The upper one-third of each ROI was automatically selected (dotted lines) from which the mean echo intensity was calculated.
Bi, biceps brachii muscle; Br, brachialis muscle; H, humerus; Ff, forearmflexors; R, radius; U, ulna;, Rf, rectus femoris muscle; Vi, vastus intermedius muscle; Ta, tibialis anterior muscle; Ti, Tibia.

Muscle thickness was measured online with electronic calipers at standardized positions.[8] The biceps brachii muscle was measured between the bone echo of the humerus and the superficial fascia of the biceps (which includes the brachialis muscle); the forearm flexors between the interosseous membrane (next to the radius) and the ventral fascia of the most ventral flexors; the quadriceps muscle between the bone echo of the femur and the superficial fascia of the rectus femoris (which includes the rectus femoris and vastus intermedius); the tibialis anterior muscle between the interosseous membrane (next to the tibia) and the ventral fascia of the tibialis anterior muscle.

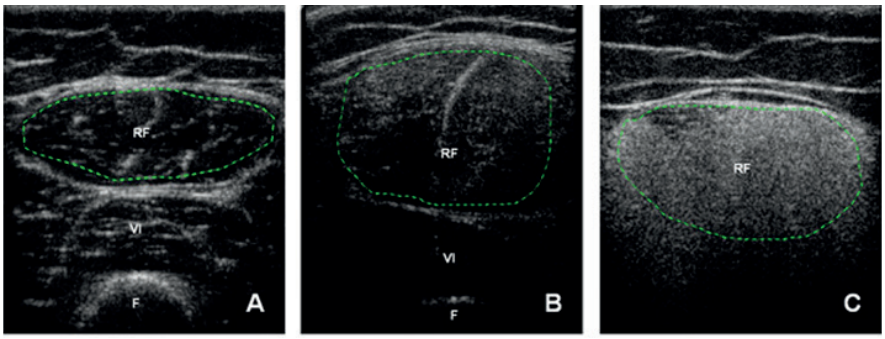

Figure 2 Echo intensities for a healthy boy and boys with DMD. Transverse scan of the rectus femoris muscle of a healthy boy (**A**), an ambulant young boy with DMD (**B**), and a wheelchair-dependent teenage boy with DMD (**C**).

The healthy 5-year old boy showed a normal echo intensity (z-score 0.1), whereas the 4.6 years old young boy with DMD showed already a mildly increased echo intensity (z-score 3.0). The teenage boy with DMD (10.6 years old) showed a strongly increased echo intensity (z-score 7.6) with an absent of the bone echo. RF, rectus femoris muscle; VI, vastus intermedius muscle; F, femur. Dotted line represents the border of the rectus femoris muscle, which was the region of interest for the calculation of the mean echo intensity.

Data analysis

Statistical analyses were performed using SPSS v. 16.0 for Windows (SPSS Inc., Chicago, IL, USA). A *p*-value of <0.05 was considered to be significant.

Muscle thickness was first corrected for weight as described in a previous study by Scholten et al. (2003).[8] Echo intensity did not need to be corrected for age, weight or sex, as it was found to be independent of these variables until the age of 18 years.[8] QMUS results (echo intensity and muscle thickness) were compared to muscle specific reference values and expressed as z-scores. Z-scores represent the number of standard deviations from the mean. They were calculated by subtracting the reference value from the measured value and then dividing the difference by the standard deviation of the reference value for that patient.[25] Analyses were done for the composite sum scores (sum z-scores biceps brachii muscle, forearm flexors, rectus femoris muscle and tibialis anterior muscle) as well as for the individual muscles (z-scores) separately. A Kolmogorov Smirnoff test confirmed that all ultrasound parameters showed a normal distribution ($p = 0.20$).

Analysis of age related changes

First it was investigated whether the ultrasound parameters changed significantly with age. This was performed using a linear Generalized Estimating Equation analysis (GEE).[26,27] GEE uses all available data for each subject and corrects for observations made at different

time intervals. Additionally, GEE corrects for the dependency of the observations from a single participant. A regression coefficient (β) was calculated, which expresses the relationship between the longitudinal development of the outcome variable (the ultrasound parameters) and the corresponding predictor variable (age per year). Therefore, β reflects the amount of change per year. In an additional GEE analysis, an age squared term (age^2) was added to the GEE analysis in order to investigate whether a ceiling effect with age exists.[27] Such a ceiling was expected to exist, as completely dystrophic muscles cannot further degenerate.

Second, of the parameters that showed a significant increase with age in the GEE analysis, we analysed whether a significant change could be found over a 1 year time period, to evaluate if that parameter could be used in a treatment trial that would run for 1 year. This was performed using a Standardized Response Mean analysis (SRM), which evaluates the changes in QMUS at a 1 year interval by dividing the mean score by the standard deviation of the change scores. SRM values of 0.2, 0.5 and 0.8 represent small, moderate and large values of responsiveness, respectively.[28,29] Of each participant the first two assessments with one year interval that included both QMUS and clinical measurements were selected.

Relation between QMUS and other clinical parameters

Longitudinal relations between QMUS and disease severity and physical functioning were also examined using GEE, in which β reflects the relationship between the development of the ultrasound parameters and the corresponding clinical parameters. SRM analysis was performed to see if a significant change over a year could be observed for any of the clinical parameters.

Cross sectional data analysis

To make our results comparable with a previous study[13] we also derived cross-sectional data from the first assessment of each participant that included both clinical measurements and muscle ultrasound during the study period. An independent *t*-test was used to compare the echo intensities of ambulant and wheelchair-dependent boys. Correlations between QMUS and age, disease severity and physical functioning were calculated using Pearson correlation coefficients (r) for continuous data and Spearman correlation coefficients (ρ) for ordinal data.

Results

Study population characteristics

Longitudinal follow-up data were available for 18 DMD boys; 15 ambulatory and 3 wheelchair-dependent, between the age of 3.7 and 15.1 years (median 8) (Table 1). All (but one) boy used corticosteroids (Prednisone) in a 10 days on 10 days off schedule of 0.75 mg/kg

Table 1 Characteristics of the participants

	n	First assessment	*n*	Final assessment
Demographics				
Age (mean±SD (range))	19	8.36±3.00 (3.72 – 15.10)	18	10.21±2.49 (6.26 – 15.20)
Clinical assessements				
Functional status (%)	19		18	
- Ambulant		15 (79)		12 (67)
- Wheelchair-dependent		4 (21)		6 (33)
Median Vignos (range)	8	5 (2 -10)	13	2 (1 – 10)
Median Brooke (range)	8	2 (1 - 5)	13	1 (1 – 5)
Mean muscle strength (mean z-scores) (SD)				
- Elbow flexor (left side)	7	-4.40 (2.28)	11	-4.20 (1.84)
- Knee extensor (right side)	6	-3.86 (0.71)	11	-3.18 (1.25)
Median CMAS (range)	7	27 (1 – 40)	13	25 (0 – 39)
Median HMAS (range)	7	28 (0 – 35)	13	26 (0 – 30)
Quantitative skeletal muscle ultrasound measures				
Mean echo intensity (mean z-scores) (SD)				
- Composite sum score	19	14.28 (8.64)	18	18.60 (8.28)
- Biceps brachii muscle	19	3.06 (2.16)	18	3.92 (2.57)
- Tibialis anterior muscle	19	3.13 (1.95)	18	4.52 (2.02)
- Rectus femoris muscle	19	5.29 (2.45)	18	6.30 (1.98)
- Forearm flexors	19	2.80 (2.69)	17	3.66 (2.36)
Mean muscle thickness (mean z-scores) (SD)				
- Composite sum score	16	1.61 (6.49)	12	1.98 (3.82)
- Biceps brachii muscle	19	0.11 (2.27)	16	-0.31 (1.49)
- Tibialis anterior muscle	19	1.19 (1.88)	16	0.39 (1.69)
- Rectus femoris muscle	16	-0.56 (2.71)	13	-0.28 (2.25)
- Forearm flexors	18	0.73 (1.47)	17	1.7 (1.12)

CMAS, Childhood Myositis Assessment Scale; HMAS, Hammersmith Motor Ability Scale

during the study period. A total of 63 ultrasound measurements were performed with a median of 3.5 measurements per boy (range 2-5). Every ultrasound exam, including the positioning of the patient and transducer, and the performance of three scans of each muscle, could be performed in less than 20 min. An additional 10 min were needed for the manual selection of the ROI's of each patient, the calculation of the mean echo intensity and muscle thickness and report generation. Median follow-up was 27.5 months (interquartile range (IQR) 22.5-32) with a median interval between two consecutive measurements of 11 months (IQR 8-12).

Echo intensity and ambulatory status were assessed during all 63 measurements. Combined muscle thickness of the rectus femoris and vastus intermedius in the quadriceps muscle could not be assessed in 25% of the ultrasound measurements because the severity of the dystrophic changes and accompanying attenuation made it impossible to visualize the bone echo even when a 8-5 MHz transducer was used instead of a 17-5 MHz transducer. Clinical assessments within 3 months of the ultrasound measurements were available for 59 to 70% of the measurements (Vignos in 70%; Brooke in 68%; elbow flexor dynamometry in 67%; knee extensor dynamometry in 59%; HMAS in 68%; CMAS in 70%).

Quantitative muscle ultrasound changes with age

Muscle echo intensities of all individual muscles as well as the composite sum score significantly increased with age (Table 2). The sum score increased with 2.2 SD per year

Table 2 The longitudinal relationship between QMUS and age

	Echo intensity		Muscle thickness	
	Assessments included	**β (95%CI)**	**Assessments included**	**β (95%CI)**
Composite sum score	64	2.24 (1.52 ; 2,97)*	46	-.48 (-1.04 ; .07)
Biceps brachii muscle	64	.49 (.31 ; .67)*	60	-.24 (-.34 ; -.14)*
Forearm flexors	64	.64 (.42 ; .86)*	61	-.05 (-.19 ; .08)
Rectus femoris muscle	64	.50 (.25 ; .75)*	48	-.16 (-.41 ; .09)
Tibialis anterior muscle	64	.60 (.38 ; .82)*	57	.08 (-.09 ; .24)

Regression coefficients (β) and 95% confidence intervals (95% CI) regarding the longitudinal relationship between quantitative skeletal muscle ultrasound (z-scores) and age as derived from the GEE analyses.
*****$p < .05$
QMUS, Quantitative muscle ultrasound

($p < 0.01$) (Figure 3). For individual muscle groups, the echo intensity of the rectus femoris muscle was already abnormal (above + 2 SD) in 50% of the boys aged 4-6 years (mean z-score = 2.7 SD ± 1.34), whereas the forearm flexors were still normal in all of these boys (mean z-score 0.10 SD ± 1.56). Above the age of 10 years, the echo intensity of the forearm flexors still increased (mean z-score in boys aged 8-10 years: 2.8 SD ± 1.8, and in boys aged >10 years: 6.6 SD ± 1.2) (Table 3). This is also reflected by the GEE analysis of the individual muscles: the strongest increase was found for the most distal muscles (forearm flexors ($\beta = 0.64$, $p < 0.001$) and tibialis anterior ($\beta = 0.60$, $p < 0.01$) muscles), as these muscle were near normal at young age. Conversely, we found a lesser increase with age for the proximal

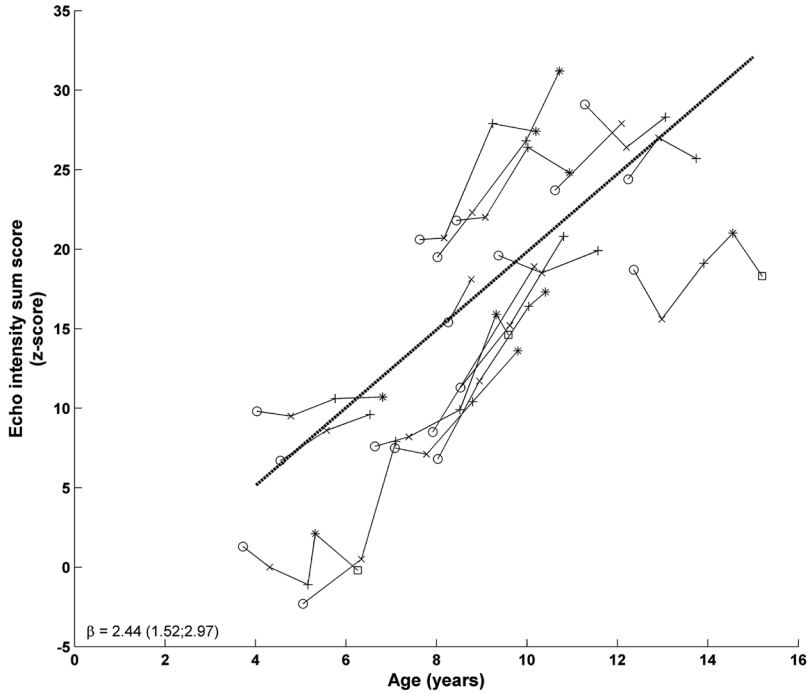

Figure 3 The longitudinal relationship between echo intensity and age.

Developmental echo intensity sum score curves are presented for each participant (first to fifth assessments are visualized as following: o, x, +, ✳, □) together with the predicted curve (dotted line). Regression coefficient (β) and 95% confidence interval are derived from the GEE analyses. Although there are some differences between the participants, the echo intensity sum score increased with age in our group of DMD participants.

muscles (biceps brachii ($\beta = 0.49$, $p < 0.01$) and rectus femoris ($\beta = 0.50$, $p < 0.01$) muscles) (Table 2), as these muscles had already higher echo intensity at young age.

Although it appeared from the data plots (Figure 3) that a ceiling effect might be present in the echo intensity after the age of twelve, this ceiling effect (age^2) was not significant ($p = 0.76$) in our cohort.

Muscle thickness of the biceps brachii decreased significantly with age ($\beta = -0.24$, $p < 0.01$). The other muscles did not show a significant decrease. (Table 2)

The SRM analysis showed good responsiveness over a one year follow up period for the echo intensity sum score (SRM = 0.77), echo intensity of the rectus femoris (SRM = 0.89) and tibialis anterior muscle (SRM = 0.79). This was comparable to the responsiveness of the clinical assessments (Table 4). Low to moderate responsiveness was found for the

Table 3 Echo intensities per age group

Echo intensity (mean±SD[range])	**Age 4-6y** (*n*=4)	**Age 6-8y** (*n*=4)	**Age 8-10y** (*n*=6)	**Age >10y** (*n*=3)
Composite sum score	3.88±5.41 (-2.30-9.80)	11.05±6.38 (7.50-20.60)	15.73±5.75 (6.80-21.80)	25.73±2.94 (23.70-29.10)
Biceps brachii muscle	0.53±1.13 (-1.00-1.60)	2.50±1.85 (0.70-5.10)	3.32±1.71 (0.60-4.90)	5.53±1.08 (4.30-6.30)
Tibialis anterior muscle	0.60±1.63 (-0.90-2.50)	2.58±0.64 (2.00-3.50)	3.55±1.10 (2.00-4.60)	5.23±1.74 (3.90-7.20)
Rectus femoris muscle	2.65±1.34 (1.50-4.40)	4.53±2.8 (2.80-8.50)	6.08±1.67 (3.60-8.10)	8.33±1.01 (7.60-9.60)
Forearm flexors	0.10±1.56 (-1.90-1.80)	1.45±1.44 (0.20-3.50)	2.78±1.83 (-0.40-4.60)	6.63±1.15 (5.50-7.80)

Echo intensities are presented in z-scores.

Table 4 Responsiveness statistics of QMUS and the clinical assessments

	n	**Mean change after 1 year follow-up (SD)**	**SRM**
Clinical assessments			
Vignos	11	0.45 (1.37)	0.33
Brooke	11	0.09 (0.30)	0.30
Muscle strength (z-scores)			
- Elbow flexor	11	-1.30 (1.23)	1.06
- Knee extensor	10	-0.61 (0.87)	0.70
CMAS	11	-3.82 (4.94)	0.77
HMAS	10	-2.80 (3.39)	0.83
Echo intensity (z-scores)			
- Composite sum score	15	2.54 (3.29)	0.77
- Biceps brachii muscle	15	0.41 (1.16)	0.35
- Tibialis anterior muscle	15	0.81 (1.03)	0.79
- Rectus femoris muscle	15	0.79 (1.01)	0.89
- Forearm flexors	15	0.52 (1.43)	0.36

QMUS, Quantitative muscle ultrasound ; SRM, Standardized Response Mean; CMAS, Childhood Myositis Assessment Scale, HMAS, Hammersmith Motor Ability Scale

echo intensity of the upper extremity muscles (BB: SRM = 0.35; FF: SRM = 0.36) and the Vignos (SRM = 0.33) and Brooke (SRM = 0.30) functional grading scores. As shown in table 5, the echo intensity of the lower extremity has good responsiveness in boys aged 4-8 years old, whereas the echo intensity of the upper extremity shows moderate responsiveness in boys older than 8 years.

Quantitative muscle ultrasound in relation to clinical assessments

Increased echo intensities of the biceps brachii and rectus femoris muscles co-occurred with a decrease in strength of the elbow flexors and knee extensors, a decrease in functional status (Vignos and Brooke scales) and lower motor ability (CMAS and HMAS scores); see table 6 and figure 4. With respect to the functional status scales, echo intensities varied per Vignos and Brooke grade and increases in echo intensity were often not matched directly by higher functional grades (Figure 4).

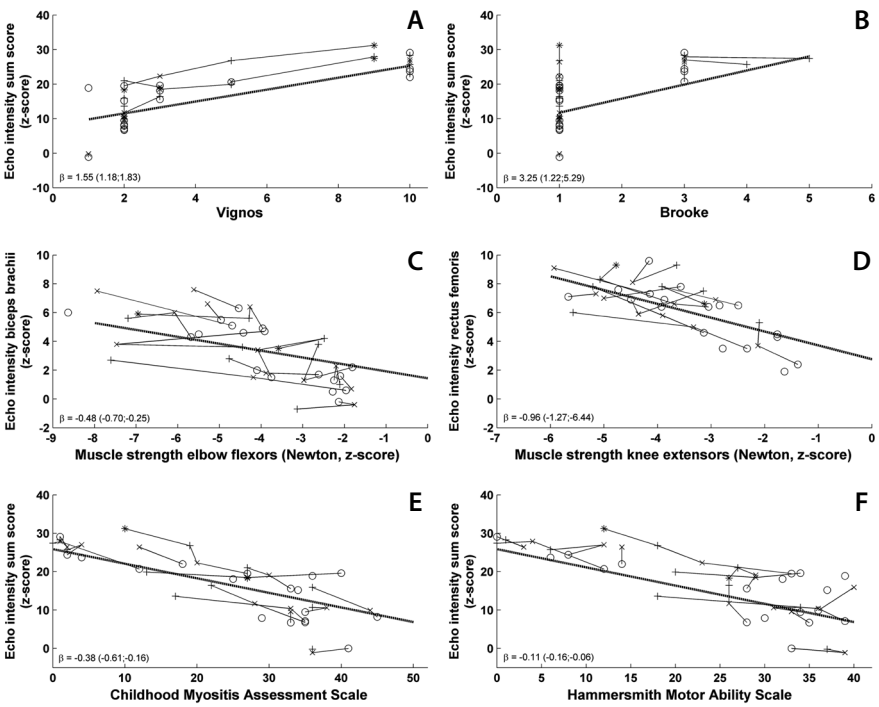

Figure 4 Echo intensity versus the clinical assessments.

Developmental echo intensity curves versus the Vignos (**A**) and Brooke (**B**) functional grades, muscle strength of the elbow flexors (**C**) and knee extensors (**D**), CMAS (**E**) and HMAS (**F**) are presented for each participant (first to fifth assessments are visualized as following: o, x, +, ⁎, □) together with the predicted curves (dotted lines). Regression coefficients (β) and 95% confidence intervals are derived from the GEE analyses. An increased echo intensity of the biceps brachii and rectus femoris muscles was related to a decreased muscle strength of the elbow flexors and knee extensors, respectively. Furthermore, an increased echo intensity sum score was related to a lower score on the CMAS and HMAS, indicating a lower motor ability.

Table 5 Responsiveness statistics of the echo intensity per age group

	Boys <8 years old			Boys ≥8years old		
	n	Mean change after 1 year follow-up (SD)	SRM	*n*	Mean change after 1 year follow-up (SD)	SRM
Echo intensity (z-scores)						
- Composite sum score	6	2.18 (3.16)	0.69	9	2.78 (3.53)	0.79
- Biceps brachii muscle	6	-0.15 (0.88)	0.17	9	0.79 (1.21)	0.65
- Tibialis anterior muscle	6	0.95 (0.40)	0.95	9	0.72 (1.32)	0.55
- Rectus femoris muscle	6	1.00 (1.05)	0.95	9	0.66 (1.03)	0.64
- Forearm flexors	6	0.38 (1.43)	0.27	9	0.61 (1.51)	0.40

SRM, Standardized Response Mean

Additional cross-sectional data

Additional cross-sectional data were available for 19 boys, of whom 18 used corticosteroids. Cross-sectional correlations between QMUS and age and the clinical assessment were comparable to the results from our longitudinal study. Echo intensities of both the composite sum score ($r = 0.69$, $p < 0.001$) and the individual muscles (BB: $r = 0.62$, $p = 0.05$; FF: $r = 0.76$, $p < 0.001$; RF: $r = 0.50$, $p = 0.03$; TA: $r = 0.64$, $p = 0.003$) were positively correlated with age, which means that older boys showed higher echo intensities than younger boys. Muscle thickness was not correlated with age after the standard correction for the bodyweight related increase in muscle thickness during childhood[8] (Table 7).

In relation to the clinical assessments, we found that wheelchair-dependent boys had a significant higher echo intensity (mean sum score = 24.1 SD ± 3.1) than ambulant boys (mean sum score = 12.9 SD ± 6.6) ($p = 0.01$). Furthermore, the sum score of the echo intensity correlated with all clinical assessments: positive correlations with the Vignos ($\rho = 0.85$, $p < 0.001$) and Brooke ($\rho = 0.71$, $p = 0.01$) functional grades, and negative correlations with muscle strength (elbow flexors: $r = -0.74$, $p < 0.01$; knee extensors: $r = -0.73$, $p = 0.01$), the CMAS ($\rho = -0.69$, $p = 0.02$) and the HMAS ($\rho = -0.66$, $p = 0.04$) scores were found. As shown in figure4, all wheelchair-dependent boys (Vignos grade 10) had composite sum z-scores of > 20 SD. For ultrasound of individual muscle groups we found significant positive correlations between the echo intensity of the rectus femoris ($\rho = 0.68$, $p = 0.02$) and tibialis anterior ($\rho = 0.74$, $p < 0.01$) muscles and the Vignos functional grade, and between the echo intensity of the biceps brachii muscle ($\rho = 0.65$, $p = 0.02$), forearm flexors ($\rho = 0.66$, $p = 0.02$) and the Brooke functional grade. Negative correlations were found between the echo intensity of the biceps brachii muscle and the muscle strength of the elbow flexors ($r = -0.77$, $p < 0.01$), and also between the echo intensity of the quadriceps

Table 6 The longitudinal relationship between echo intensity and the clinical assessments

	Assessments included	β (95%CI)
Echo intensity composite sum score		
Vignos	42	1.55 (1.18 ; 1.83)*
Brooke	42	3.25 (1.22 ; 5.29)*
CMAS	43	-.38 (-.61 ; -.16)*
HMAS	42	-.39 (-.53 ; -.24)*
Echo intensity Biceps brachii muscle		
Vignos	42	.41 (.27 ; .55)*
Brooke	42	1.10 (.49 ; 1.72)*
Muscle strength elbow flexors	42	-.48 (-.70 ; -.25)*
CMAS	43	-.11 (-.16 ; -.06)*
HMAS	42	-.11 (-.15 ; -.07)*
Echo intensity Forearm flexors		
Vignos	42	.49 (.39 ; .59)*
Brooke	42	1.10 (.60 ; .1.62)*
CMAS	43	-.12 (-.18 ; -.05)*
HMAS	42	-.11 (-.15 ; -.07)*
Echo intensity Rectus femoris muscle		
Vignos	42	.30 (.14 ; .45)*
Brooke	42	.46 (-.45 ; 1.37)
Muscle strength knee extensors	37	-.96 (-1.27 ; -6.44)*
CMAS	43	-.11 (-.16 ; -.06)*
HMAS	42	-.11 (-.14 ; -.08)*
Echo intensity tibialis anterior muscle		
Vignos	42	.38 (.23 ; .54)*
Brooke	42	.66 (.25 ; 1.08)*
CMAS	43	-.09 (-.13 ; -.04)*
HMAS	42	-.10 (-1.45 ; -.54)*

Regression coefficients (*β*) and 95% confidence intervals (95% CI) regarding the longitudinal relationship between quantitative echo intensity (z-scores) and the clinical assessments as derived from the GEE analyses.
*$p < .05$
CMAS, Childhood Myositis Assessment Scale; HMAS, Hammersmith Motor Ability Scale

muscle and the muscle strength of the knee extensors ($r = -0.79$, $p < 0.01$). Echo intensity of the quadriceps muscle correlated negatively with the CMAS ($\rho = -0.71$, $p = 0.001$) and HMAS ($\rho = -0.68$, $p = 0.03$) scores (Table 7).

With respect to the muscle thickness, only the thickness of the quadriceps muscle correlated negatively with the muscle strength of the knee extensors ($r = -0.73$, $p = 0.03$), and no significant correlations were found for the sum score and other individual muscles.

Table 7 Correlations between QMUS and age and the clinical assessments

	Age*	Vignos**	Brooke**	Muscle strength*		CMAS**	HMAS**
				EF	KE		
Echo intensity							
Composite sum score	0.69	0.85	0.71	-0.74	-0.73	-0.69	-0.66
Biceps brachii muscle	0.62	0.76	0.65	-0.77	-0.72	-0.58	-0.52
Tibialis anterior muscle	0.64	0.74	0.55	-0.56	-0.63	-0.50	-0.48[#]
Rectus femoris muscle	0.50	0.68	0.49	-0.75	-0.79	-0.71	-0.68
Forearm flexors	0.76	0.82	0.66	-0.69	-0.62	-0.75	-0.64

Correlation coefficients were derived from the cross-sectional study. Correlation coefficients are given in Pearson correlation coefficients (r)* for continuous data and in Spearman correlation coefficients (ρ)** for ordinal data. All correlations (except one[#]) were significant at the $p \leq 0.05$ level. Correlations between muscle thickness and age and the clinical assessments are not shown as none of them, except the correlation between the muscle thickness of the quadriceps muscle and muscle strength of the knee extensors ($r = 0.73$, $p < .05$), was significant.
QMUS, Quantitative muscle ultrasound; EF, elbow flexors; KE, knee extensors; CMAS, Childhood Myositis Assessment Scale; HMAS, Hammersmith Motor Ability Scale

Discussion

This study shows that echo intensity assessed with quantitative muscle ultrasound has significant relations with age and clinical parameters including functional grading scores, muscle strength, ambulatory status and motor ability. It establishes quantitative muscle ultrasound (QMUS) as a practical, quick, child-friendly and feasible tool for longitudinal follow up in Duchenne muscular dystrophy, not influenced by the patients' condition, fatigability or cooperation.

Our results demonstrate that in the youngest boys aged 4-6 years the echo intensity of the rectus femoris was already clearly abnormal (SD > 2) in 2 of the 4 boys while the echo intensity of the other muscles measured (biceps brachii, forearm flexors and tibialis anterior) was often still in the normal range. Conversely, the most distal muscles in our protocol still show a significant increase in echo intensity even after the age of 10 years. However, as most of the boys assessed in our study were 6-10 years old, extending these results to younger and older boys with DMD should be done with caution and requires further research. Nevertheless, the echo intensity sum score of the four muscles in our protocol increased in every age group with 2.2 SD per year. Our findings corroborate earlier evidence that echo intensities are higher in more severely affected, older, boys with DMD.[13]

All muscles examined showed good responsiveness, indicating that one has a good chance of finding a significant difference in echo intensity when repeating the measurement after 1 year. We found the highest responsiveness values for the echo

intensity sum score, rectus femoris and tibialis anterior muscle. The responsiveness to change of these muscle echo intensities was comparable to measures of muscle strength and motor function, and better than the coarser categories of functioning used in the Vignos and Brooke scales. Further research is recommended to investigate the relationship between QMUS and the 6MWT, which is the primary outcome measure in many clinical trials in boys with DMD.[30] The responsiveness to change of the echo intensity of the upper extremity muscles was slightly less in our total cohort, but was also good in boys older than 8 years. This is in accordance with the fact that proximal muscles are affected later in DMD than the distal muscles.[19]

Combined, our findings mean that muscle echo intensity is sensitive to the progressive changes in muscle architecture in DMD patients between 4 and 12 years, even when they use corticosteroids and even with the current set of four muscles not specifically chosen for DMD. It would be interesting to study whether other muscles, such as the gastrocnemius and proximal arm muscles (e.g. deltoid) are even more responsive to changes over time in boys who are going through life-events such as the transition from walking to wheelchair use. For example, the gastrocnemius muscle could be useful for ambulant boys, as MRI studies have shown that posterior compartments of the lower leg are affected earlier than anterior compartments (e.g. the tibialis anterior muscle).[31,32] The deltoid muscle might be useful for wheelchair-dependent boys who start experiencing difficulties in reaching and lifting with their arms.

Counter-intuitively we found no signs of generalized disuse atrophy over time in our population of DMD boys. We expected atrophy as both cross-sectional morphometric and magnetic resonance imaging studies have shown that atrophy of severely affected muscles (such as the adductor magnus and rectus femoris) progresses with age.[33,34] However, only the muscle thickness of the biceps brachii decreased significantly with age in our longitudinal analysis and the other muscles did not. We did find a slight decrease in muscle thickness of the quadriceps muscle at baseline, but apparently this atrophy was not progressive. A previous cross-sectional ultrasound study also showed atrophy of the quadriceps without abnormalities in thickness in the other muscles in DMD patients compared to healthy subjects.[7] A limitation of studying quadriceps muscle thickness with ultrasound is that with progression of the disease abnormalities it becomes impossible to visualize the bone echo of the femur because of the attenuation of ultrasound waves in the dense fibrotic and fatty tissue (Figure 2).[35] In this study the thickness of the combined rectus femoris and vastus intermedius muscles could not be assessed in 25 % of the measurements because of this effect, even when a lower frequency transducer was used.

Data from DMD boys older than 12 years (shown in Figure 3) indicate that a ceiling effect might be present with respect to muscle echo intensity. Such a ceiling effect has been found in other measures of muscle strength and clinical functioning[19], and was also

expected for echo intensity as muscles in DMD can only degenerate up to a certain point before they have become fully dystrophic and all muscle tissue has been replaced by fat and fibrosis. Although our data also indicated a possible ceiling effect, this was not significantly present in any of the four muscles assessed. This lack of significance might be explained by the small number of boys in the age group of 12 years and older, or , alternatively, it might mean that no significant ceiling effect is present in this age group, as such a ceiling effect was also not found in an earlier cross sectional study on muscle echo intensity of the elbow flexors in DMD patients up to 19 years.[13] It deserves further investigation in different age-groups, including both children and young adult DMD patients, to establish which muscle group will reach this echo intensity ceiling at which point in the disease course. As most new therapies will likely and preferably be tested in the younger DMD patients, the possible presence of a ceiling effect at the end of the second decade of life will probably have limited practical consequences for using QMUS as a follow up tool in treatment trials.

Although our results show that QMUS can quantify and follow muscle changes during the course of DMD, and as such would be very suited as a practical and patient-friendly follow-up tool, its implementation in different centers is currently complicated by the need for specific normal values that have to be established for every different ultrasound machine. Although this can be achieved by using degradable phantoms (i.e. meat) with a conversion method described earlier[24] or establishing one's own normal values, these methods are a bit cumbersome and new developments in ultrasound technology are necessary that focus on the device-independent interpretation of echo intensities. Until then, we recommend the use of one and the same ultrasound device and settings for which normative data are available, especially when the QMUS technique is used as part of a clinical trial.

In conclusion, our results show that QMUS is a responsive outcome measure to assess disease severity in the follow-up of boys aged 4-12 years with DMD. As it is quick, non-invasive and reflects underlying pathological changes without the need for muscle biopsy and patient cooperation we recommend its use in future treatment trials and daily clinical care.

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References

1. McDonald CM, Henricson EK, Han JJ et al. The 6-minute walk test as a new outcome measure in Duchenne muscular dystrophy. *Muscle Nerve* 2010;41:500-510.
2. Mercuri E, Mayhew A, Muntoni F et al. Towards harmonisation of outcome measures for DMD and SMA within TREAT-NMD; report of three expert workshops: TREAT-NMD/ENMC workshop on outcome measures, 12th--13th May 2007, Naarden, The Netherlands; TREAT-NMD workshop on outcome measures in experimental trials for DMD, 30th June--1st July 2007, Naarden, The Netherlands; conjoint Institute of Myology TREAT-NMD meeting on physical activity monitoring in neuromuscular disorders, 11th July 2007, Paris, France. *Neuromuscul Disord* 2008;18:894-903.
3. Scott OM, Hyde SA, Goddard C, Dubowitz V. Quantitation of muscle function in children: a prospective study in Duchenne muscular dystrophy. *Muscle Nerve* 1982;5:291-301.
4. Beenakker EA, Maurits NM, Fock JM, Brouwer OF, van der Hoeven JH. Functional ability and muscle force in healthy children and ambulant Duchenne muscular dystrophy patients. *Eur J Paediatr Neurol* 2005;9:387-393.
5. Cotton SM, Voudouris NJ, Greenwood KM. Association between intellectual functioning and age in children and young adults with Duchenne muscular dystrophy: further results from a meta-analysis. *Dev Med Child Neurol* 2005;47:257-265.
6. Darke J, Bushby K, Le Couteur A, McConachie H. Survey of behaviour problems in children with neuromuscular diseases. *Eur J Paediatr Neurol* 2006;10:129-134.
7. Pillen S, Verrips A, Alfen van N, Arts IM, Sie LT, Zwarts MJ. Quantitative skeletal muscle ultrasound: diagnostic value in childhood neuromuscular disease. *Neuromuscul Disord* 2007;17:509-516.
8. Scholten RR, Pillen S, Verrips A, Zwarts MJ. Quantitative ultrasonography of skeletal muscles in children: normal values. *Muscle Nerve* 2003;27:693-698.
9. Maurits NM, Beenakker EA, van Schaik DE, Fock JM, van der Hoeven JH. Muscle ultrasound in children: normal values and application to neuromuscular disorders. *Ultrasound Med Biol* 2004;30:1017-27.
10. Reimers K, Reimers CD, Wagner S, Paetzke I, Pongratz DE. Skeletal muscle sonography: a correlative study of echogenicity and morphology. *J Ultrasound Med* 1993;12:73-77.
11. Heckmatt JZ, Leeman S, Dubowitz V. Ultrasound imaging in the diagnosis of muscle disease. *J Pediatr* 1982;101:656-660.
12. Heckmatt J, Rodillo E, Doherty M, Willson K, Leeman S. Quantitative sonography of muscle. *J Child Neurol* 1989;4 Suppl:S101-S106.
13. Zaidman CM, Connolly AM, Malkus EC, Florence JM, Pestronk A. Quantitative ultrasound using backscatter analysis in Duchenne and Becker muscular dystrophy. *Neuromuscul Disord* 2010;20:805-809.
14. Arts IM, Pillen S, Schelhaas HJ, Overeem S, Zwarts MJ. Normal values for quantitative muscle ultrasonography in adults. *Muscle Nerve* 2010;4:32-41.
15. Goodpaster BH, Carlson CL, Visser M et al. Attenuation of skeletal muscle and strength in the elderly: The Health ABC Study. *J Appl Physiol* 2001;90:2157-2165.
16. Maurits NM, Bollen AE, Windhausen A, De Jager AE, van der Hoeven JH. Muscle ultrasound analysis: normal values and differentiation between myopathies and neuropathies. *Ultrasound Med Biol* 2003;29:215-25.
17. Brooke MH, Griggs RC, Mendell JR, Fenichel GM, Shumate JB, Pellegrino RJ. Clinical trial in Duchenne dystrophy. I. The design of the protocol. *Muscle Nerve* 1981;4:186-197.
18. Florence JM, Pandya S, King WM et al. Clinical trials in Duchenne dystrophy. Standardization and reliability of evaluation procedures. *Phys Ther* 1984;64:41-45.
19. McDonald CM, Abresch RT, Carter GT et al. Profiles of neuromuscular diseases. Duchenne muscular dystrophy. *Am J Phys Med Rehabil* 1995;74(5 Suppl):S70-S92.
20. Beenakker EA, van der Hoeven JH, Fock JM, Maurits NM. Reference values of maximum isometric muscle force obtained in 270 children aged 4-16 years by hand-held dynamometry. *Neuromuscul Disord* 2001;11:441-446.
21. Mayhew JE, Florence JM, Mayhew TP et al. Reliable surrogate outcome measures in multicenter clinical trials of Duchenne muscular dystrophy. *Muscle Nerve* 2007;35:36-42.

22. Lovell DJ, Lindsley CB, Rennebohm RM et al. Development of validated disease activity and damage indices for the juvenile idiopathic inflammatory myopathies. II. The Childhood Myositis Assessment Scale (CMAS): a quantitative tool for the evaluation of muscle function. The Juvenile Dermatomyositis Disease Activity Collaborative Study Group. *Arthritis Rheum* 1999;42:2213-2219.
23. Huber AM, Hicks JE, Lachenbruch PA et al. Validation of the Childhood Health Assessment Questionnaire in the juvenile idiopathic myopathies. Juvenile Dermatomyositis Disease Activity Collaborative Study Group. *J Rheumatol* 2001;28:1106-1111.
24. Pillen S, van Dijk JP, Weijers G, Raijmann W, de Korte CL, Zwarts MJ. Quantitative gray-scale analysis in skeletal muscle ultrasound: a comparison study of two ultrasound devices. *Muscle Nerve* 2009;39:781-786.
25. Duffy ME, Munro BH, Jacobsen BS. Key Principles of Statistical Inference. In: Munro BH, editors. *Statistical methods for health care research*. 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2005, p 73-107.
26. Laird NM, Ware JH. Random-effects models for longitudinal data. *Biometrics* 1982;38:963-974.
27. Twisk JWR. *Applied longitudinal data analysis for epidemiology*. Cambridge: Cambridge University Press; 2003.
28. Husted JA, Cook RJ, Farewell VT, Gladman DD. Methods for assessing responsiveness: a critical review and recommendations. *J Clin Epidemiol* 2000;53:459-468.
29. Cohen J. *Statistical power analysis for the behavioral sciences*. 2nd ed. New Jersey: Lawrence Erlbaum Associates;1977.
30. Goemans NM, Tulinius M, van den Akker JT et al. Systemic administration of PRO051 in Duchenne's muscular dystrophy. *N Engl J Med* 2011;364:513-522.
31. Kinali M, rechavala-Gomeza V, Cirak S et al. Muscle histology vs MRI in Duchenne muscular dystrophy. *Neurology* 2011;76:346-353.
32. Schreiber A, Smith WL, Ionasescu V et al. Magnetic resonance imaging of children with Duchenne muscular dystrophy. *Pediatr Radiol* 1987;17:495-497.
33. Marden FA, Connolly AM, Siegel MJ, Rubin DA. Compositional analysis of muscle in boys with Duchenne muscular dystrophy using MR imaging. *Skeletal Radiol* 2005;34:140-148.
34. Cros D, Harnden P, Pellissier JF, Serratrice G. Muscle hypertrophy in Duchenne muscular dystrophy. A pathological and morphometric study. *J Neurol* 1989;236:43-47.
35. Pillen S, Arts IM, Zwarts MJ. Muscle ultrasound in neuromuscular disorders. *Muscle Nerve* 2008;37:679-93.

3

The Assisted Six-Minute Cycling Test to assess endurance in children with a neuromuscular disorder

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Abstract

Introduction: For late or non-ambulant children with a neuromuscular disorder no suitable endurance tests are currently available. We developed the Assisted Six-Minute Cycling Test (A6MCT) for the legs and arms and investigated its psychometric properties in healthy boys and boys with Duchenne muscular dystrophy (DMD).

Methods: Ninety-nine healthy boys and 30 boys with DMD (12 wheelchair-dependent) performed the A6MCT. Seventy healthy boys additionally performed the Six-Minute Walk Test (6MWT), and 23 boys performed the A6MCT twice within 2 weeks. Boys with DMD also performed the Motor Function Measure (MFM).

Results: The A6MCT was feasible for > 90% of all boys. Boys with DMD achieved less cycling revolutions than controls. The A6MCT was positively correlated with the 6MWT and was reproducible in healthy boys, and it correlated with disease severity in boys with DMD.

Discussion: The A6MCT is a promising outcome measure for the follow-up of non-ambulant children with a neuromuscular disorder.

Introduction

Neuromuscular disorders (NMD) in childhood are heterogeneous, but all have the general feature of progressive loss of muscle function. Although there is no cure for most NMD yet, promising treatments are now becoming available.[1] To monitor disease progression and the effect of such new treatments,[2] clinically meaningful outcome measures related to children's abilities to function in daily life are needed. Endurance tests are often used as such an outcome measure in many clinical trials in children with NMD,[1] because they have been proven to correlate with functioning in daily life.[3, 4]

Endurance exercise protocols for children with NMD can be divided into maximal ("all-out") and submaximal tests.[5] During "all-out" tests, children are motivated to reach their maximal mechanical power and aerobic peak, i.e. the highest volume of oxygen that can be consumed by the body per time unit. Such a maximum test is often not feasible for children with NMD, who cannot reach their aerobic peak capacity or maximum heart rate because of muscle weakness and local fatigue. A submaximal test that predicts rather than directly assesses the maximal exercise capacity is better suited for this group and has the additional advantages of being safer, more comfortable and less exhausting.

Currently, the most frequently used submaximal test for children with NMD is the Six-Minute Walk Test (6MWT)[3] that assesses the distance that a patient can quickly walk without running in six minutes. The 6MWT is easy to perform and feasible for ambulatory children with NMD[6], although its responsiveness to measure changes over time might be limited as shown by relatively large standard deviations of the within-subject change in walked distance.[7] However, for children with progressive NMD who are at the end of their ambulatory phase or are already wheelchair-dependent, no suitable submaximal endurance test is currently available. This is problematic for long-term follow up, and in a time that controlled treatment trials are also being developed for these older, more severely impaired patients there is a need for a feasible and reliable clinical outcome measure.

The aim of our study was to develop a submaximal endurance test for both the legs (leg cycling) and arms (arm cranking) for children who are expected to lose their walking ability in the near future as well as children who have recently become wheelchair-dependent and have problems with lifting and reaching with their arms. We chose to develop a cycling test for both the arms and legs that can be performed by both ambulatory and wheelchair-confined children and has the additional advantage that participants are less at risk for falls. Although regular bicycle ergometers have the ability to adjust their mechanical power to the capacity of children with severe muscle weakness, the initial load and further increments are often still too hard to sustain for these patients, for when the bicycle ergometer displays no resistance the child is still cycling at more than 5 Watts.[5]

We therefore chose to use a motor-assisted device that would allow even very weak patients to perform the movements required for the tests.

In the first part of this paper we describe the development of our Assisted Six-Minute Cycling Test (A6MCT), a bicycle test for the legs and arms using a motor-assisted mobility trainer. In the second part we investigated the feasibility of the A6MCT in healthy boys and boys with Duchenne muscular dystrophy (DMD), which is the most common inherited muscular dystrophy affecting young boys and results in wheelchair-dependency at a mean age of ten years.[8] Girls were not recruited to participate in this study, since DMD primarily affects males. For healthy boys, we also examined the relationship between the A6MCT and the commonly used 6MWT (construct validity), and we investigated whether the same test results are obtained when the test is repeated within a short period under the same circumstances (test-retest reliability). For boys with DMD, we investigated the relationship between the A6MCT and disease severity.

Methods

This study was approved by the Medical Ethics Committee Arnhem-Nijmegen, The Netherlands. All parents, and participants who were over 12 years of age, gave their written informed consent.

Part 1: Development of the A6MCT

Rationale and device

The A6MCT was developed in collaboration with several experts from the neuromuscular center of the Radboud University Nijmegen Medical Centre, including two rehabilitation physicians, one physiotherapist, three neurologists and clinical neurophysiologists and two biomedical engineers. For the motor-assisted bicycle ergometer device we chose to use a mobility trainer (KPT Cycla, Kinetic, France) that could be used with the patients' personal (electric) wheelchair if necessary, so the patient did not need to be transferred to a regular home trainer. The decision for the specific type of mobility trainer used in our validation study was based on the availability of motor assistance, the constant assistance and resistance provided, the stability of the device on the floor layer and the possibility to easily alternate between leg and arm testing. Motor assistance was expected to allow for bicycle testing even when muscle strength was insufficient to achieve fully active movements. Furthermore, the device could be adapted to body height and the pedal distance could also be adjusted to limb length.

Duration and assistance

We aimed to develop a submaximal test (heart rate about 70% of the max heart rate) for both the legs and arms in a single-stage protocol. We chose a protocol length of 6 minutes in which children were free to choose their own speed, to match the commonly used 6MWT which has been shown to be feasible and relevant for children with NMD.[7] A protocol length of more than 6 minutes could diminish the chance of a suboptimal performance due to short attention span in some children.

In order to determine a feasible level of resistance (Wmax) for children with severe muscle weakness, we conducted a pilot study in three boys with DMD (two community walkers aged 9 and 10 years, and one household walker aged 9 years). Participants cycled in periods of 1 minute with a continuous speed of 65 rounds per minute (RPM) at several resistance levels ranging from level 1 (7.7 Watt) to level 10 (51.3 Watt). The same procedure was used for both legs and arms. A feasible resistance level was defined as a level that could be sustained for one minute cycling at 65 RPM without exhaustion. Overall physical fatigue was assessed using the OMNI scale for perceived exertion that grades the perceptions of physical exertion on a scale from 0 (not tired at all) to 10 (very very tired) and contains both verbal and pictorial descriptions.[9]

Results from the first part of this pilot study showed that boys with DMD were able to cycle with both legs and arms with a continuous speed of 65 RPM only at the lowest resistance level (7.7 Watt) for 1 minute without becoming exhausted (OMNI scale score ≤ 6). However, in the second part of the pilot study, we asked the boys to continue cycling at their own speed for another six minutes, and they all had to terminate the test already after approximately 3 minutes. Therefore, we decided to adjust the protocol and to use fixed motor assistance (passive mode 1, no load speed 7RPM) instead of resistance. With this protocol, the boys were able to complete 6 minutes of cycling at their own constant velocity. Our pilot study among the 3 boys with DMD confirmed the feasibility of this A6MCT for both legs and arms.

Final A6MCT protocol

The Assisted Six-Minute Cycling Test (A6MCT) for the legs and arms was performed using the motor assisted mobility trainer (KPT Cycla, Kinetic, France) in passive mode 1 with a no-load speed of 7RPM for every participant. After a short demonstration the participants were instructed to cycle as fast as possible and keep this up for 6 minutes. Starting positions for both the leg cycling and arm cranking protocols were standardized; see figure 1. For the A6MCT for the legs, the hip and knee of the bended leg were held in ~90° flexion, while the knee of the other leg was submaximally extended. For the A6MCT of the arms the pedal axis was a few centimeters (with a maximum of 5 cm) below shoulder level when the pedals were horizontal. The distance from the chair to the bicycle was determined by allowing participants to move their legs and arms over the submaximal

range of motion, which was produced a feeling of stretch but not pain (Figure 1). Participants were seated comfortably with the back supported by the back of the seat. Verbal encouragements from the instructor to maintain attention and to complete the test as well as possible were given every 15 s throughout the exercise (Appendix 1). The assessor was seated next to the participant and informed the participant about the time completed and left, and about the amount of revolutions cycled so far. Participants were allowed to rest if they were not able to continue cycling due to fatigue. In that case, they were also motivated to continue cycling as soon as possible. The primary test outcome was the number of revolutions achieved in 6 minutes. Revolutions per minute (cumulative) and resting periods were also recorded.

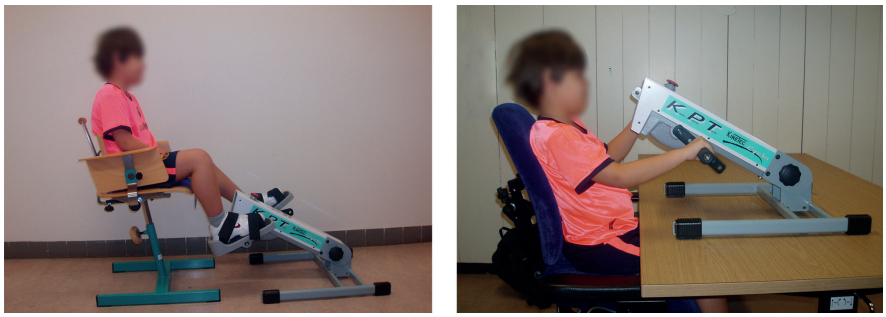

Figure 1 Starting position of the Assisted Six-Minute Cycling Test for the legs and arms.

This figure has been previously published in M. Jansen et al. (2010) [10]. Permission was obtained from the original publisher, BioMedCentral, to reproduce the figure.

Part 2: Validation of the A6MCT in healthy boys

Participants

Healthy boys, without limitations in arm or leg function, aged 6-16 years were recruited from three Dutch primary schools from May 2010 to April 2011. Age (years), height (m) and body weight (kg) were recorded.

Feasibility

Healthy boys from the three different schools performed the A6MCT for the legs and arms and cycled at passive mode 1 with a no-load speed of 7RPM. The tests for the legs and arms were performed in random order to exclude bias due to fatigue. Participants rested ten minutes in a chair prior to each test to recover from the expected submaximal effort and to start each test with a resting heart rate. Heart rate (beats per minute, bpm) was

assessed using a standard heart rate monitor (Onyx classic, Sigma, Germany) prior and after the A6MCT. We also assessed the perceived overall physical exertion using the OMNI scale for perceived exertion (see above: Duration and assistance) and recorded any signs of exercise intolerance (i.e. excessive muscle pain, extreme fatigue, dizziness, or an uncomfortable feeling). All assessments took place at the primary schools of the participants and were conducted by four researchers (MdJ, HC, FE, and MJ).

Construct validity

A subgroup of healthy boys from two primary schools also performed the Six-Minute Walk Test (6MWT) in addition to the A6MCT in order to examine the relationship between both endurance tests. The 6MWT was performed according to an adjusted protocol for DMD patients, as described by McDonald et al (2010).[7] Participants were instructed to walk as fast as possible without running. The assessor walked ~1.5 m behind the participant and encouraged the participant every 15 s to keep exercising using standardized phrases. As the corridors of the primary schools involved in this study were only 20 m long, participants performed the 6MWT in a corridor with a marked test area of 20 m instead of the usual 25 m. Participants were allowed to rest or to stop if they were not able to continue. The distance walked at the end of the six-minute period was recorded in meters, together with the cumulative distance walked (per minute), rest periods needed and number of falls.

Test-retest reliability

To study the test-retest reliability of the A6MCT, boys from the third primary school performed the A6MCT for the legs and arms twice with a two-week interval. Participants and researchers involved in this part of the study were blinded from previous test results during the second test day.

Part 3: Validation of the A6MCT in boys with DMD

Participants

For validation of the A6MCT in Duchenne Musucular Dystrophy (DMD) we obtained test results from age-matched boys with DMD from the randomized controlled No Use is Disuse (NUD) study.[10] We used only the baseline data from this study, meaning that participants had not yet received any physical training intervention. The NUD study included boys with DMD who were at the end of their ambulatory phase (needing ≥ 5 s to get up from the floor, unable to get up from the floor, unable to bicycle without assistance, and dependent on a wheelchair to move over a distance > 500 m), or had recently (within 1-2 years prior) begun full time use of a wheelchair. Full time users of a wheelchair, in this study defined as wheelchair-dependent boys, had to be able to touch

the top of their head with both hands, or had to be able to use a hand-operated wheelchair, indicating a moderate-to-good arm-hand function. Age (years), height (m) and body weight (kg) were registered.

Feasibility

Procedures were similar to those described for healthy boys, and all boys intended to perform both the A6MCT for the legs and arms. However, in the NUD study the test order was standardized, and boys first performed the A6MCT for the legs and then for the arms. Furthermore, all assessments were performed by one researcher (MJ) and took place at the Department of Rehabilitation of the Radboud University Nijmegen Medical Centre.

Relationship between the A6MCT and disease severity

The boys with DMD also performed the Motor Function Measure (MFM) as part of the NUD study, and the baseline results were used to assess the relationship between the A6MCT and disease severity. The MFM is a valid and reliable scale to assess motor function in both ambulant and wheelchair-dependent patients with a neuromuscular disorder.[11] The scale consists of 32 items in 3 dimensions: Dimension 1, Standing position and transfers (D1); Dimension 2, Axial and proximal motor function (D2); Dimension 3, Distal Motor Function (D3). Each item is scored on a 4-point Likert scale (generic grading: 0, does not initiate movement or starting position cannot be maintained; 1, partially completes the exercise; 2, completes the exercise with compensations, slowness or obvious clumsiness; 3, completes the exercise with a standard pattern). The scale has a maximum score of 96, and a higher score indicates better motor function. We calculated a percentage (%) of the maximum score for the total MFM score.

Statistical analysis

Statistical analyses were performed using SPSS v. 16.0 for Windows (SPSS Inc., Chicago, IL, USA). A p-value of ≤0.05 was considered to be significant.

Performance and feasibility

Performance on the A6MCT was described as mean ± standard deviation (SD) for continuous data (number of revolutions achieved and heart rate) and as median with range for ordinal data (OMNI scale) for healthy boys, the total group of boys with DMD, ambulatory boys with DMD and wheelchair-dependent boys with DMD. Healthy boys and boys with DMD, and also ambulatory and wheelchair-dependent boys with DMD, were compared using independent t-tests for continuous data (age, height, body weight, A6MCT, heart rate) and Mann-Whitney U tests for ordinal data (OMNI scale). The feasibility of the A6MCT for the legs and arms was determined by calculating the percentage of boys who were able to perform the test. We deemed that the test was feasible when at least 95% of the healthy boys and 90% of the boys with DMD would be able to perform

the A6MCT. The correlation between the A6MCT and age was investigated by calculating Pearson correlation coefficients (*r*) for both healthy boys and boys with DMD. Pearson correlation coefficients were interpreted as following: 0 to 0.25 - little to any correlation, 0.26 to 0.49 - low correlation, 0.50 to 0.69 - moderate correlation, 0.70 to 0.89 - high correlation and correlations ≥0.90 - very high correlation.[12]

Construct validity in healthy boys

The relationship between the A6MCT and the 6MWT was examined by calculating Pearson correlation coefficients (*r*) and using stepwise linear regression analysis, as parametric normal distributions were confirmed by the Kolmogorov-Smirnoff test ($p > 0.05$). Pearson correlation coefficients were interpreted as described above.[12] Regression coefficients (β) and 95% confidence intervals (95% CI) were calculated from stepwise linear regression analysis. The 6MWT was the only independent variable in step one of the regression analysis, whereby the A6MCT was the dependent variable. Height was added as potential confounder in the second step of the regression analysis, since the 6MWT is positively related to height in healthy boys.[7] We expected a moderate-to-high positive correlation between the A6MCT and the 6MWT, as both tests aim to measure submaximal endurance.

Test-retest reliability in healthy boys

The test-retest reliability of the A6MCT (legs and arms) was first explored by examining whether any changes existed between the first and second assessment using paired *t*-tests. Next, intraclass correlation coefficients (ICC) were calculated and Bland-Altman figures including 95% limits of agreement ($Mean_{Difference} \pm 1.96 * SD_{Difference}$) were generated.[13, 14] ICCs were considered acceptable when they were above 0.70.[15]

Relationship of the A6MCT with disease severity in boys with DMD

The correlation between the A6MCT and disease severity as expressed by the MFM was examined by calculating Spearman correlation coefficients (rho). Correlation coefficients were calculated for the total group of boys with DMD, but also separately for ambulatory and wheelchair-dependent boys. We expected a low-to-moderate positive correlation, as both outcome measures were expected to assess different aspects of disease severity (i.e. endurance versus motor function).

Results

Validation of the A6MCT in healthy boys

Participant characteristics

Ninety-nine healthy boys with a mean age of 9.9 years participated in this study. Characteristics of the healthy boys are shown in table 1.

Feasibility and performance

Of the 99 healthy boys included in this study, 95 performed the A6MCT for the legs, and another 95 performed the A6MCT for the arms. The reasons for missing data were mainly a lack of time to perform both tests. Only one boy was unable to complete the A6MCT for the legs, because his feet tended to slip off the pedals. This means that the A6MCT was feasible for 99% of the healthy boys, without any signs of exercise intolerance.

The mean number of revolutions achieved during six minutes of cycling was 843 ± 82 for the legs and 778 ±111 for the arms (Table 1). Figure 2 shows the constant cycling velocity (rounds per minute) which participants selected and maintained during the A6MCT for the legs and arms. Figure 3 shows the positive correlation between the number of revolutions achieved with the A6MCT for the legs and the arms (r = 0.64, p < 0.01), indicating that boys who achieved a high number of revolutions with the arms also achieved a high number of revolutions with the legs and vice versa. The mean heart rate

Table 1 Subject characteristics and test results of healthy boys and boys with Duchenne muscular dystrophy

	n	Healthy (n=99) Mean ± SD (range)	n	DMD (n=30) Mean ± SD (range)	
Demographics					
Age (y)	98	9.9 ± 2.0 (6.4 ; 16.8)	30	10.5 ± 2.6 (6.4 ; 16.6)	p = 0.02*
Body weight (kg)	98	35.0 ± 8.9 (18.0 ; 63.7)	30	45.5 ± 19.4 (18.3 ; 92.4)	p < 0.01*
Height (m)	98	1.4 ± 0.1 (1.2 ; 1.9)	30	1.4 ± 0.2 (1.1 ; 1.8)	p < 0.01*
A6MCT (revolutions)					
Legs	95	843 ± 82 (604 ; 1016)	29	405 ± 152 (118 ; 714)	p < 0.01*
Arms	95	778 ± 111 (492 ; 1003)	28	370 ± 120 (142 ; 574)	p < 0.01*
6MWT (distance)	70	623 ± 72 (411 ; 829)	NA	NA	NA
MFM (%)	NA	NA	30	66.2 ± 14.2 (32.3 ; 91.7)	NA

* = Significant difference between healthy boys and boys with DMD at the 0.05 level.
DMD, Duchenne muscular dystrophy; A6MCT, Assisted Six-Minute Cycling Test; 6MWT, Six-Minute Walk Test; NA, Not assessed; MFM, Motor Function Measure

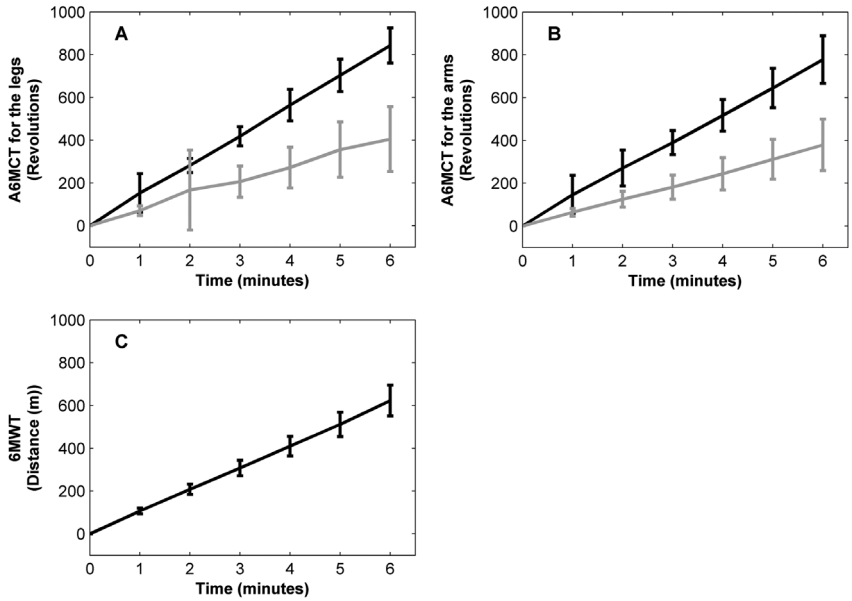

Figure 2 Cumulative number of revolutions for the legs (**A**) and arms (**B**) assessed using the A6MCT, and cumulative distance (m) assessed using the 6MWT (**C**) per minute.

Mean and SD are shown. Black lines represent healthy boys. Grey lines represent boys with Duchenne muscular dystrophy.
A6MCT, Assisted Six-Minute Cycling Test ; 6MWT, Six-Minute Walk Test

prior to the A6MCT was 95 bpm for the legs and 96 bpm for the arms, and increased to a maximum of 161 bpm and 163 bpm, respectively (Table 2). Perceived exertion increased from 0 (not tired at all) to 4 (getting more tired) on a maximal scale of 0 (not tired at all) to 10 (very very tired) during both the A6MCT for the legs and arms. As expected, the number of revolutions achieved with the A6MCT for the legs ($r = 0.38$, $p < 0.01$) and arms ($r = 0.60$, $p < 0.01$) increased moderately with age in healthy boys.

Construct validity

Seventy healthy boys performed the A6MCT for both the legs and arms and the 6MWT. Two boys were excluded; one of them fell during the 6MWT and walked carefully thereafter (no injuries occurred), and another boy ran instead of walking. The mean walked distance at the 6MWT was 623 ± 72 m, with a max heart rate of 160 bpm and an OMNI scale for perceived exertion score of 3 (Table 2). Heart rate and OMNI scale scores at the end of both the A6MCT and 6MWT were comparable (Table 2).

Table 2 Subject characteristics and test results of healthy boys and boys with Duchenne muscular dystrophy

	Healthy	DMD	Significance
A6MCT legs			
HR start	95.4 ± 12.3	100.5 ± 12.3	$p = 0.11$
HR end	161.0 ± 21.5	155.4 ± 17.9	$p = 0.27$
OMNI start	0 (0 ; 6)	1 (0 ; 3)	$p = 0.07$
OMNI end	4 (0 ; 10)	6 (0 ; 10)	$p = 0.03^*$
A6MCT arms			
HR start	95.9 ± 12.0	107.28 ± 12.3	$p < 0.01^*$
HR end	163.0 ± 22.3	149.3 ± 17.5	$p < 0.01^*$
OMNI start	0 (0 ; 10)	2 (0 ; 8)	$p < 0.01^*$
OMNI end	4 (0 ; 10)	6 (0 ; 10)	$p = 0.02^*$
a6MWT			
HR start	100.0 ± 13.9	NA	NA
HR end	160.1 ± 20.4	NA	NA
OMNI start	0 (0 ; 7)	NA	NA
OMNI end	3 (0 ; 10)	NA	NA

* = Significant difference between healthy boys and boys with DMD.
A6MCT, Assisted Six-Minute Cycling Test; 6MWT, Six-Minute Walk Test; HR, heart rate; OMNI, OMNI scale for perceived exertion; DMD, Duchenne muscular dystrophy; NA, Not assessed

The A6MCT for the legs ($r = 0.58$, $p < 0.01$) and the A6MCT for the arms ($r = 0.65$, $p < 0.01$) were moderately correlated with the 6MWT (Figure 4). This means that boys who walked further during the 6MWT also achieved a higher number of revolutions with the A6MCT compared to boys who walked a shorter distance.

Results from the linear regression analysis confirmed the positive relationship between the A6MCT for the legs ($\beta = 0.6$, $p < 0.01$) and the arms ($\beta = 1.0$, $p < 0.01$) and the 6MWT. After correction for height, the results were slightly different (A6MCT for the legs: $\beta = 0.7$, $p < 0.01$, A6MCT for the arms: $\beta = 0.7$, $p < 0.01$). Height was also positively related to the A6MCT for the arms ($p = 0.01$), indicating that tall boys achieved more revolutions with their arms than small boys, but not to the A6MCT for the legs ($p = 0.08$).

Test-retest reliability

Twenty-three (leg cycling) and twenty-two (arm cranking) boys performed the A6MCT twice within two weeks. With respect to A6MCT for the legs, the mean number of revolutions was 870 ± 93 for the first assessment and 875 ± 92 for the second assessment (mean difference = 4 ± 46, $p = 0.70$). For the A6MCT for the arms, boys achieved on average 33 ± 46 revolutions more during the second assessment (mean number of revolutions = 840 ± 118) compared to the first assessment (mean number of revolutions = 807 ± 117)

(p = < 0.01). Nevertheless, a high degree of correlation was found between the two assessments for both the legs (ICC = 0.88, 95% CI = 0.72 ; 0.95) and arms (ICC= 0.89, 95% CI = 0.76 ; 0.95). Bland-Altman plots confirmed the good test-retest reliability of the A6MCT for both the legs and arms by showing that only 1 or 2 data points (4.3 – 9.1%) were beyond the 95% limits of agreement and by showing that the difference in the number of revolutions achieved between the first and second assessment was not correlated with the mean number of revolutions for the two tests (legs: r = -.04, p = 0.87; arms: r = -0.31, p = 0.18) (Figure 5).

Validation of the A6MCT in boys with DMD

Participant characteristics

Thirty boys with DMD (18 ambulant, 12 wheelchair-dependent) with a mean age of 10.6 years were included in this study. Characteristics of the total group of boys with DMD are shown in table 1 and in table 3 for ambulatory and wheelchair-dependent boys separately. As expected, wheelchair-dependent boys were older and taller than ambulatory boys (p < 0.01).

Feasibility and performance

Of the thirty boys with DMD who participated in this study, 29 (97%) performed the A6MCT for the legs, as one wheelchair-dependent boy, with an MFM score of 47%, had insufficient muscle strength, particularly of the hip flexors, to be able to perform the test. Of the other 29 boys who were able to perform the A6MCT for the legs, 93% (i.e. 27 of the 29 boys), had an MFM score of ≥ 50%. The two boys who scored less than 50% at the MFM (32 and 41% respectively), achieved a relatively low number of revolutions with their legs (< 225). Another two boys did not perform the A6MCT for the arms due to problems with attention span. All other boys (100%) were able to complete the A6MCT for the arms. No periods of rest were needed during the tests, and no signs of exercise intolerance were reported.

As expected, boys with DMD achieved less revolutions with their legs (mean number of revolutions = 405 ± 152) and arms (mean number of revolutions = 370 ± 120) compared to healthy controls with only little overlap (p < 0.01) (Table 1). However, boys with DMD also cycled at a constant velocity throughout the tests (Figure 2), and the correlation between the A6MCT for the legs and arms was comparable as for healthy boys (Figure 3). Regarding the endurance parameters (Table 2), heart rates and OMNI scale scores for perceived exertion prior to the A6MCT for the arms were increased in DMD boys compared to healthy controls. After the A6MCT for the legs, the heart rate response (maximum of 155 bpm) and increase in OMNI scale scores (mean OMNI score = 6) in DMD boys were comparable to healthy controls. Maximum heart rate at the end of the A6MCT for the arms was slightly lower in boys with DMD (maximum of 149 bpm) compared to healthy boys (p < 0.01). No signs of exercise intolerance were observed. The number of revolutions

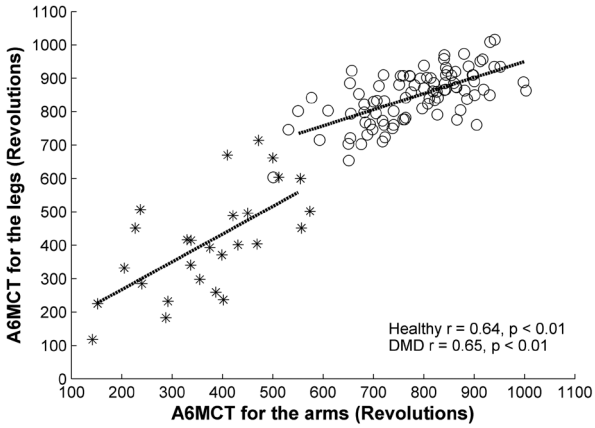

Figure 3 Correlations between the A6MCT for the legs (**A**) and arms (**B**).

The A6MCT for the legs and arms are positively correlated in both healthy boys (open circles) and boys with DMD (stars).
A6MCT, Assisted Six-Minute Cycling Test; DMD, Duchenne muscular dystrophy

achieved with the A6MCT for the legs(r = -0.18, p = 0.34) and arms (r = 0.22, p = 0.25) was not significantly correlated with age and was not significantly different for ambulatory and wheelchair-dependent boys (Table 3).

Table 3 Subject characteristics and test results of ambulatory and wheelchair-dependent boys with Duchenne muscular dystrophy

	n	**Ambulatory (n=18)** Mean ± SD (range)	n	**Wheelchair (n=12)** Mean ± SD (range)	
Demographics					
Age (y)	18	9.3 ± 1.7 (6.4 ; 11.6)	12	12.4 ± 2.6 (8.7 ; 16.6)	$p < 0.01$*
Body weight (kg)	18	33.6 ± 8.6 (18.3 ; 49)	12	63.3 ± 17.3 (35.7 ; 92.4)	$p < 0.01$*
Height (m)	18	135.0 ± 13.6 (114 ; 166)	12	158.3 ± 16.2 (131 ; 181)	$p < 0.01$*
A6MCT (revolutions)					
Legs	18	431.44 ± 147.9 (183 ; 714)	11	361.6 ± 154.4 (118 ; 670)	$p = 0.24$
Arms	16	371.88 ± 110.8 (205 ; 555)	12	368.3 ± 135.6 (142 ; 574)	$p = 0.94$
MFM (%)	18	75.0 ± 9.3 (54.2 ; 91.7)	12	52.8 ± 8.5 (32.3 ; 61.5)	$p < 0.01$*

* = Significant difference between healthy boys and boys with DMD at the 0.05 level.
DMD, Duchenne muscular dystrophy; A6MCT, Assisted Six-Minute Cycling Test; 6MWT, Six-Minute Walk Test; NA, Not assessed; MFM, Motor Function Measure

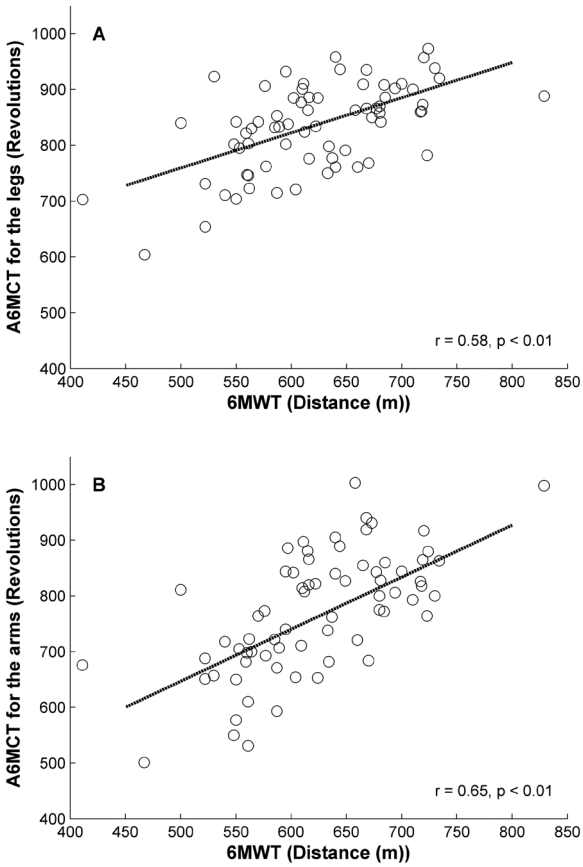

Figure 4 Correlations between the 6MWT and the A6MCT for the legs (**A**) and arms (**B**) in healthy boys.

The A6MCT for the legs and the A6MCT for the arms are positively correlated with the 6MWT. A6MCT, Assisted Six-Minute Cycling Test; 6MWT, Six-Minute Walk Test

Relationship with disease severity

The mean MFM score (percentage) was 66.2 ± 14.2 (range 32.3 – 91.7). Wheelchair-dependent boys (mean MFM % = 52.8 ± 8.5) had lower MFM scores than ambulatory boys (mean MFM % = 75.0 ± 9.3) ($p < 0.01$, Table 3). As shown in figure 6, the number of revolutions achieved with the A6MCT for the legs was positively correlated with the MFM in the total group of boys with DMD ($\rho = 0.65$, $p < 0.01$), the ambulatory boys ($\rho = 0.72$, $p < 0.01$), and the wheelchair-dependent boys ($\rho = 0.74$, $p = 0.01$). This means that the number of revolutions

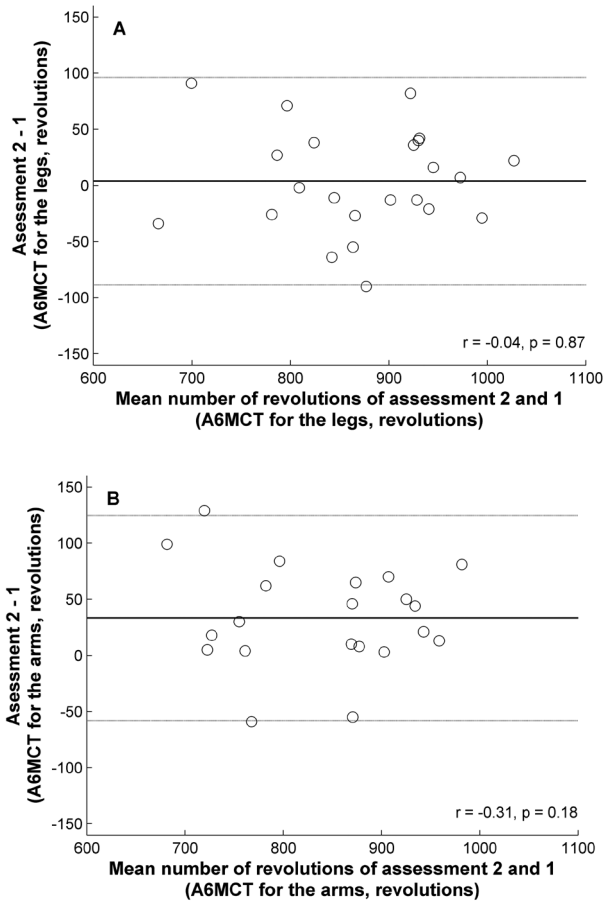

Figure 5 Bland-Altman plots for the A6MCT for the legs (**A**) and arms (**B**) with the mean (solid line) and 95% limits of agreement (dotted lines) for comparisons between assessment 1 and 2 in healthy boys.

A6MCT, Assisted Six-Minute Cycling Test

achieved with the A6MCT for the legs decreased with a decrease in motor function. A similar positive correlation between the A6MCT for the arms and motor function was found for wheelchair-dependent boys ($\rho = 0.84$, $p < 0.01$). A trend for a positive correlation between the A6MCT for the arms and motor function was also found for the the total group of DMD patients ($\rho = 0.32$, $p = 0.94$) and for ambulatory boys ($\rho = 0.46$, $p = 0.07$), but this was not significant. (Figure 6)

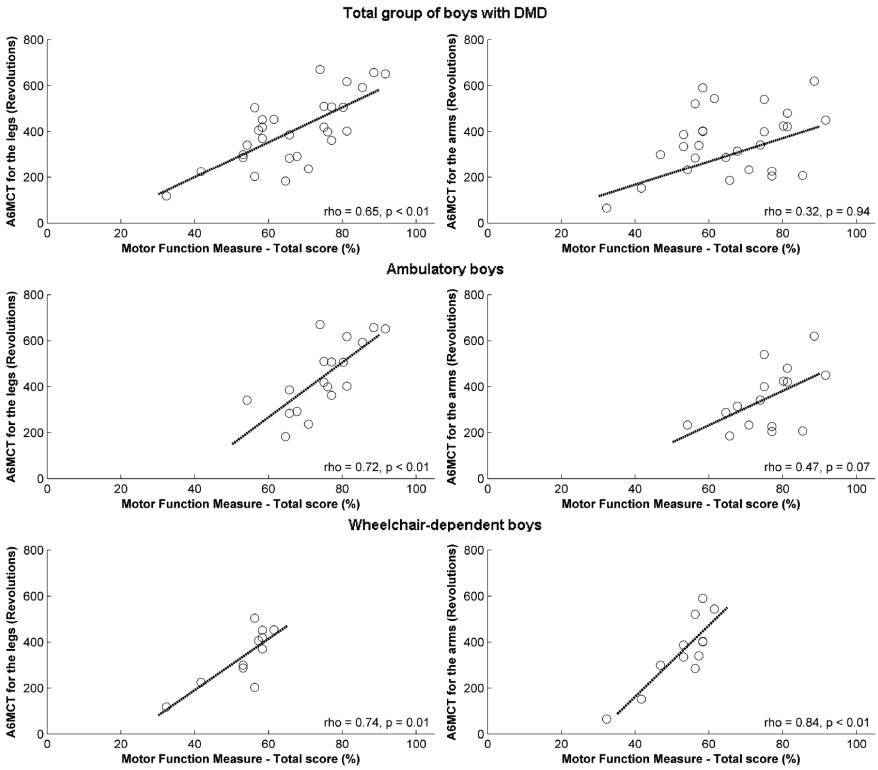

Figure 6 Correlations between the A6MCTfor the legs and arms and the total score of the Motor Function Measure (%) in boys with DMD.

A6MCT, Assisted Six-Minute Cycling Test

Discussion

This study shows that the Assisted Six-Minute Cycling Test (A6MCT) for the legs and arms is a feasible, valid and reproducible endurance test for children and adolescents aged 6-16 years. The A6MCT is the first ergometer test that can also be performed by wheelchair-dependent children with a progressive neuromuscular disorder and shows comparable characteristics of endurance testing in healthy boys. The motor assistance enables children with severe muscle weakness to perform a submaximal endurance test that is related to their disease severity.

Our results demonstrate that the A6MCT with a fixed motor assistance level (no-load, speed 7RPM) is feasible for both healthy boys and boys with Duchenne muscular dystrophy (DMD). Ninety-nine percent of the healthy boys and 97% of the boys with DMD were able to perform the A6MCT for the legs. Our preliminary results suggest that the threshold value for being able to perform the A6MCT for the legs is a total MFM score of around 50% for boys with DMD. With respect to the A6MCT for the arms, all healthy boys and boys with DMD were able to perform the test. This is unique, as other ergometer tests are often too hard to sustain for children with severe muscle weakness due to their initial load and increments in resistance.[16,17]

The A6MCT provided a sensitive outcome measure to distinguish between healthy boys and boys with DMD in this study. Boys with DMD achieved significantly fewer revolutions with their legs and arms compared to healthy age-matched controls. There was very little overlap in performance; just 2 boys with DMD performed within 2 SD of the mean number of revolutions achieved by healthy boys for both the legs and arms. Our findings corroborate earlier evidence that boys with DMD have a lower functional capacity than healthy boys.[7]

Although wheelchair-dependent boys achieved slightly fewer revolutions with the A6MCT than ambulatory boys with DMD, we did not find a significant correlation with age as was shown by previous studies on the 6MWT.[7] The absence of a negative correlation between the A6MCT and age in our study could be explained by the relatively heterogeneous phenotype of DMD in our study, i.e. we included ambulatory as well as wheelchair-dependent patients. Even within these two groups, we found a large variability in motor function with age. For example, 2 8-year old boys participated in this study; one was ambulatory and was relatively fit, whereas the other boy was already confined to a wheelchair. Previous studies also confirmed the heterogeneity of the DMD phenotype from the motor, respiratory and survival points of view.[18] Longitudinal research is needed to investigate the responsiveness of the A6MCT.

The validity of the A6MCT was confirmed by its relationship with the often-used 6MWT in healthy boys and its correlation with disease severity as shown by the MFM in boys with DMD. Although one could speculate that the motor assistance could be unnecessary and limit the performance of healthy boys, we found that healthy boys who walked further also achieved a higher number of revolutions with the A6MCT compared to boys who walked less far. This means that the distance cycled was related to the submaximal level of functional capacity. Reproducibility data from healthy boys showed that boys achieved on average 33 revolutions more during a second assessment for the arms, which was performed within two weeks after the first assessment. This can be explained by a learning effect. Dutch boys, even those with an NMD, are accustomed to bicycling with their legs (thus being trained), but not with their arms (thus being untrained).

For boys with DMD, the distance cycled with the legs decreased with a lower motor function, indicating that the A6MCT is also able to measure clinically meaningful changes over time.

Also the data of the wheelchair-dependent boys with DMD confirmed the positive correlation between the A6MCT for the arms and the MFM. The absence of a relationship between the number cycled with the arms and the MFM within the total group of boys with DMD and the subgroup of ambulatory boys was not entirely unexpected, as both tests assess two different aspects of disease severity (i.e. endurance and motor function). Furthermore, the MFM measures proximal motor functions, but the number of items which assess gross arm motor functions is limited, and it is well known that fine arm motor functions (such as writing) are relatively preserved in boys with DMD.[19] No other arm function tests, except the practical but non-sensitive general Brooke scale for upper limb function[20], exist for DMD patients. The A6MCT for the arms could therefore become a first test for the assessment of arm function in NMD patients.

The submaximal nature of the A6MCT for the legs and arms was confirmed by the maximum heart rate of about160 bpm and the perceived exertion score of "getting more tired" in healthy boys. The relatively high start heart rates might be explained by the excitement of the healthy boys to participate in the study. Maximal heart rates were comparable to those reached during the 6MWT, and also to those reached by boys with DMD during the A6MCT for the legs (155 bpm) and arms (149 bpm). As no studies have yet reported the aerobic peak of boys with DMD, one could speculate that the A6MCT is more "all-out" for boys with DMD than for healthy boys. Maximum perceived exertion scores were only "tired" and not "very very tired", indicating that boys exercised at a submaximum level.[20] Future trials should investigate the relationship between the A6MCT, cardiac and pulmonary function. Increases in perceived exertion were comparable to healthy boys, and no signs of exercise intolerance were found, confirming the feasibility and safety of the A6MCT for boys with DMD.

Outcome measures for use in clinical trials on DMD, especially those that investigate the effectiveness of new drug treatments, should be clinically relevant and present how participants feel and function in a proper time period. With respect to the A6MCT, the meaningfulness for the boys and their parents could be limited by the fact that cycling is less critical to human performance in daily life than walking. Arm cranking, however, could be correlated with arm abilities and should be studied further. Overall, the results of this first validation study are promising and support further research on the A6MCT for children with NMD, especially in a time that treatment trials are being developed for more severely impaired NMD patients. We recommend investigation of the relationship between the A6MCT and clinically relevant endpoints (such as the age at which ambulation or the ability to put on a t-shirt are lost) and the ability of the A6MCT to measure changes

over time. Additionally, we recommend investigation of the relationship between the A6MCT and the 6MWT in a larger group of NMD patients and a feasibility study among older, even more severely disabled children and young adults with NMD. We also recommend the establishment of normative data for healthy girls, though we do not expect any gender differences from the literature on the 6MWT.[21] Finally, we recommend a practice test to reduce measurement errors due to an improvement in coordination and a reduction in anxiety.[3] Assessors using the A6MCT should always remember the influence of children's' motivation to participate in the test and adhere to the standardized procedures of encouragements.

In conclusion, our results show that the A6MCT for the legs and arms is a promising objective outcome measure to monitor disease progression and to evaluate effectiveness of treatments in children with severe muscle weakness who are either restricted walkers or wheelchair-dependent.

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References

1. Goemans NM, Tulinius M, van den Akker JT et al. Systemic administration of PRO051 in Duchenne's muscular dystrophy. *N Engl J Med* 2011; 364:1513-1522.
2. Mercuri E, Mayhew A, Muntoni F et al. Towards harmonisation of outcome measures for DMD and SMA within TREAT-NMD; report of three expert workshops: TREAT-NMD/ENMC workshop on outcome measures, 12th--13th May 2007, Naarden, The Netherlands; TREAT-NMD workshop on outcome measures in experimental trials for DMD, 30th June--1st July 2007, Naarden, The Netherlands; conjoint Institute of Myology TREAT-NMD meeting on physical activity monitoring in neuromuscular disorders, 11th July 2007, Paris, France. *Neuromuscul Disord* 2008;18:894-903.
3. ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories. ATS statement: guidelines for the six-minute walk test. *Am J Respir Crit Care Med* 2002;166:111-117.
4. Mazzone E, Martinelli D, Berardinelli A et al. North Star Ambulatory Assessment, 6-minute walk test and timed items in ambulant boys with Duchenne muscular dystrophy. *Neuromuscul Disord* 2010;20:712-716.
5. Bar-Or O, Rowland TW. Procedure for exercise testing in children. In: Bar-Or O, Rowland TW, editors. *Pediatric exercise medicine. From physiologic principles to health care application.* United States of America: Human Kinetics; 2004. p 343-365.
6. McDonald CM, Henricson EK, Han JJ et al. The 6-minute walk test in Duchenne/Becker muscular dystrophy: longitudinal observations. *Muscle Nerve* 2010;42:966-974.
7. McDonald CM, Henricson EK, Han JJ et al. The 6-minute walk test as a new outcome measure in Duchenne muscular dystrophy. *Muscle Nerve* 2010;41:500-510.
8. McDonald CM, Abresch RT, Carter GT et al. Profiles of neuromuscular diseases. Duchenne muscular dystrophy. *Am J Phys Med Rehabil* 1995;74:S70-S92.
9. Robertson RJ, Goss FL, Aaron DJ et al. Observation of perceived exertion in children using the OMNI pictorial scale. *Med Sci Sports Exerc* 2006;38:158-166.
10. Jansen M, de Groot IJM, van Alfen N, Geurts ACH. Physical training in boys with Duchenne Muscular Dystrophy: the protocol of the No Use is Disuse study. *BMC Pediatr* 2010;10:55.
11. Berard C, Payan C, Hodgkinson I, Fermanian J. A motor function measure for neuromuscular diseases. Construction and validation study. *Neuromuscul Disord* 2005;15:463-470.
12. Munro BH. Correlation. In: Munro BH, editors. *Statistical methods for health care research.* 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2005, p 239-258.
13. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986;1:307-310.
14. Bland JM, Altman DG. Measuring agreement in method comparison studies. *Stat Methods Med Res* 1999;8:135-160.
15. de Vet HC, Terwee CB, Knol DL, Bouter LM. When to use agreement versus reliability measures. *J Clin Epidemiol* 2006;59:1033-1039.
16. Bar-Or O, Rowland TW. Neuromuscular and musculoskeletal diseseases. In: Bar-Or O, Rowland TW, editors. *Pediatric exercise medicine. From physiologic principles to health care application.* United States of America: Human Kinetics; 2004. p 269-321.
17. Tirosh E, Bar-Or O, Rosenbaum P. New muscle power test in neuromuscular disease. Feasibility and reliability. *Am J Dis Child* 1990;144:1083-1087.
18. Humbertclaude V, Hamroun D, Bezzou K et al. Motor and respiratory heterogeneity in Duchenne patients: Implication for clinical trials. *Eur J Paediatr Neurol* 2011;16:149-160.
19. Wagner MB, Vignos PJ, Jr., Carlozzi C, Hull AL. Assessment of hand function in Duchenne muscular dystrophy. *Arch Phys Med Rehabil* 1993;74:801-804.
20. Robertson RJ, Goss FL, Boer NF et al. Children's OMNI scale of perceived exertion: mixed gender and race validation. *Med Sci Sports Exerc* 2000;32:452-458.
21. Priesnitz CV, Rodrigues GH, Stumpf CS et al. Reference values for the 6-min walk test in healthy children aged 6-12 years. *Pediatr Pulmonol* 2009;44):1174-1179.

Appendix 1 Assisted Six-Minute Cycling Test

General procedures

Equipment

1) Mobile trainer (KPT Cycla, Sprofit, Kinetic);
2) Stopwatch;
3) Polar band;
4) OMNI scale for perceived exertion;
5) Height adjustable chair;
6) Height adjustable table;
7) Footstool.

Set up

1) The test should be performed in a quiet room, preferably without any family or friends in the room.
2) The mobile trainer should be placed against the wall to prevent moving.
3) Participants should be seated comfortably with their back supported by the back of the seat.
4) Assessor sits next to the participant.
5) For the A6MCT for the legs, the hip and knee of the bended leg should be hold in ~90° flexion, while the knee of the other leg is submaximally extended.
6) For the A6MCT of the arms the pedal axis should be a few centimeters (with a maximum of 5 cm) below shoulder level when the pedals are horizontal.
7) The distance from the chair to the bicycle should be determined by allowing participants to move their legs and arms over the submaximal range of motion, which may cause a feeling of stretch but not pain.
8) Passive mode 1 of the mobility trainer should be used, i.e. no-load speed of 7RPM.
9) Show the number of revolutions at the mobility trainer display.

Test procedure

1) Briefly demonstrate the cycling exercise and allow a short practical test.
2) A 10 minute rest period should be given prior to the test.
3) Record heart rate and level of perceived exertion.
4) Set stopwatch to zero.
5) Read the following statement: *"You will cycle as fast as possible and keep this up for 6 minutes. Try to continue until I tell you to that you have finished. You are allowed to rest, but try to continue for the whole 6 minutes."*
6) The assessor should inform the participant about the time completed and left, and about the amount of revolutions cycled.

7) The assessor records the number of revolutions every minute.
8) The assessor should give verbal encouragements to maintain attention and to complete the test 15 s throughout the exercise.
9) Encouragements could be:
   a. *"You're doing a great job! Keep going!"*
   b. *"Wow, you're doing great! Keep going!"*
   c. *"You're doing great, only 3 minutes left! Keep going!"*
10) At the final 10 seconds of the test, the assessor should count down.
11) Record the total number of revolutions cycled, heart rated and perceived exertion at 6 minutes.

Determining a valid test

1) A test is valid if the participant completes or discontinues the test due to fatigue.
2) A test is invalid if the participant:
   a. Discontinuous the test due reasons other than fatigue (such as noncompliance, injury).
   b. Didn't follow the instructions.

Scoring sheet

Name:		Date of test:	
Date of birth:		Examiner:	
Time (minutes)	**Revolutions (cumulative)**	**Heart rate (beats per minute)**	**Fatigue (OMNI score)**
1			
2			
3			
4			
5			
6			
Number of revolutions at six minutes:		**Comments:**	

4

Health-related quality of life and its relation to disease severity in boys with Duchenne muscular dystrophy: satisfied boys, worrying parents. A case-control study

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Abstract

The progression of Duchenne muscular dystrophy (DMD) is expected to negatively influence the patients' Health Related Quality of Life (HRQoL), but knowledge of the relationship with disease severity is limited. We investigated the relationship between HRQoL (KIDSCREEN-52 questionnaire) and disease severity (clinical assessments of body functions and activities) in forty boys with DMD (19 ambulant, 21 wheelchair-dependent) who were in different phases of the disease and underwent life-limiting events such as the loss of the ability to ambulate and the ability to lift the arms. Additionally, we compared boys' HRQoL perceptions with that of their parents. HRQoL was similar to healthy peers and not influenced by disease severity, except for the physical domain. Parents scored much lower than the boys on the KIDSCREEN-52 domains "Self Perception", Moods and Emotions" and "Bullying". The latter finding needs attention in the management of DMD.

Introduction

Duchenne muscular dystrophy (DMD) is the most commonly inherited progressive muscular disorder in childhood that affects around 1/4000 live- born males. Boys with DMD become increasingly dependent in their daily activities due to progressive paresis and loss of functional abilities.[1,2] They generally become wheelchair-dependent around the age of ten years and lose arm function around the age of 15 years. In the advanced stages of the disease, cardiac or respiratory failure eventually become life- threatening.[3] As new therapies are being developed to increase the life expectancy of boys with DMD, there is an increased need for appropriate and valid outcome measures to evaluate to what extent disease and treatment affect the child's health and well-being.[4] The importance of a patients' point of view on their health status is widely recognized and increasingly used as outcome measure.[5] From this perspective, the international Food and Drug Administration (FDA) recommends the inclusion of a Health Related Quality of Life (HRQoL) assessment instrument as an outcome measure in all clinical trials.[6]

HRQoL is an individual's perception of health and one's position in life in the context of the culture and value systems in which they live in relation to their goals, expectations, standards and concerns.[7] Because it is a broad definition, there is a great variety in the assessment and interpretation of HRQoL. HRQoL questionnaires often cover only a selection of the composing factors and aspects the broad definition of HRQoL. This means that HRQoL measures do usually not assess the entire HRQoL concept. Previous studies of disease progression and HRQoL in boys with DMD show disparities in the interpretation of HRQoL measurements (e.g. the quantity and extensiveness of the questionnaires or the use of proxy-report versus child report). Some studies had similar outcomes; Davis[8], Baiardini[9] and McDonald[10] described a lower HRQoL in boys with DMD compared to healthy controls, particularly on the physical domain. Kohler[3] focused on pulmonary function, the need for mechanical ventilation and physical disability and concluded that there was no correlation between these functions and HRQoL. HRQoL in relation to disease progression and activity level has received less research attention. In addition, proxy-reports may lead to biased results as parents tend to underestimate the HRQoL perceived by their sons.[11]

In this study we explored a broad view on the HRQoL in boys with DMD in relation to disease progression, especially during the life-limiting events as losing the ability to walk and losing arm function. To study the spectrum of physical functioning as well as the functional abilities , we used the International Classification of Functioning disability and health (ICF)-model as a starting point. This model describes the "body functions" and "activities and participation" and records the impact of the environment on the person's functioning by including contextual and environmental factors.[12,13] The benefit of this classification is the inclusion of functional activities and participation, which reflect the

child's activities regardless of his or her physical abilities. In addition, the ICF provides a basis for content of outcome measures such as HRQoL.[14] A recent study using the ICF model to evaluate the HRQoL in boys with DMD found the level of participation in social activities was significantly lower after the age of 10 years, but that the HRQoL did not decrease in the older cohort.[15]

The primary aim of this study was to broadly describe HRQoL in different disease severity groups (losing the ability to walk, being wheelchair-dependent but with a good arm function, and being wheelchair dependent and losing arm function) in boys with DMD, while simultaneously assessing the agreement between them and their parents. Our second aim was to examine the correlations between HRQoL and dimensions of the ICF model in boys with DMD.

For a broad view on HRQoL we used the KIDSCREEN-52 (KS-52). The KS-52 is a new European Generic measure of HRQoL in children aged 8 to 18 years. It was recently developed and assesses many different aspects of HRQoLin ten domains. It also contains self-report forms in addition to the parent report forms.[16] There are norm values for culture/ European country, age and gender groups. The reliability and validity of the KS-52 were found adequate.[17,18]

Methods

Design and Participants

We analysed the cross-sectional baseline data of the No use is Disuse (NUD) study.[2] The NUD study investigated the effects of assisted training in boys with DMD.

The boys were distributed in three groups depending on their functional phase of the disease: boys who (1) were ambulatory; (2) had recently (1-2yrs) become wheelchair-dependent, most of the time, with good arm function, or (3) had already been wheelchair-dependent for several years (2-5yrs) with decreased arm function. This division was made with the aid of the Vignos and Brooke scales[19], which will be explained in the outcome measures. We excluded the boys below eight years from our analysis, because they were not able to complete the KS-52.

Participants visited our department of Rehabilitation and underwent the clinical assessments by one certified examiner (MJ). Both parents and boys filled out the KS-52 at home (child and proxy-version) with instructions. Parents and boys over the age of 12 gave their written informed consent. This study was approved by our local ethics committee.

Outcome Measures

The primary outcome measure of this study is the KIDSCREEN-52 (=KS-52), which is a questionnaire that consists of 52 items measuring 10 domains of HRQoL: "physical well being", "psychological well being", "moods and emotions", "self-perception", "autonomy",

"parent relation and home life", "financial resources", "social support and peers", "school environment" and "social acceptance". Each item is scored on a 5-point Likert scale (1: no agreement at all, 5: totally agree), a higher score per item indicates a better HRQoL. If a responder left more than 25% of the items unanswered in a domain, the domain was counted as a missing value.

In a previous study of the KIDSCREEN-group of Ravens-Sieberer[18] have developed reference values for a population sample from 13 European countries including the Netherlands, which consisted of 22.827 healthy children and children with "special healthcare needs". Reference values were also obtained from proxy measures for caregivers and parents. A so-called t-value of 50 (range 45 to 55) was the mean score per item; a score above 55 meant a higher HRQoL, a score below 45 meant a lower HRQoL compared to the reference group.[16] We used these reference values in our study to compare the HRQoL of boys with DMD to healthy controls.

The ICF model consists of the dimensions "body functions" and "activities and participation", which will be translated in clinimetric secondary outcome measures.

Measures of body functions were muscle strength and endurance. Muscle strength of the shoulder abductors, elbow extensors, hip extensors, knee extensors and ankle dorsal flexors was measured bilaterally using the Medical Research Council Scale *(MRC)*. The MRC is an ordinal scale that grades the muscle strength on a scale of 0-5; grade 0 means that no movement is observed, grade 5 means maximum contraction against full resistance. The Assisted Six-Minute Cycling Test *(A6MCT)* for the legs and arms was used to assess endurance. The A6MCT has been validated for DMD and assesses the number of revolutions a boy can achieve during six minutes cycling while using motor-assisted mobility training equipment.[20]

Several outcome measures were used to assess the activity-level of the boys: the Vignos and Brooke functional scales, the Motor Function Measure (MFM), the Abilhand and the Dutch Pediatric Evaluation of Disability Inventory (PEDI).

The Vignos and Brooke functional scales classify lower and upper extremity functioning of boys with DMD[19]. The Vignos scale scores the functional ambulatory status ranging from being able to walk the stairs without guardrails (0) to not being able to sit in a wheelchair (10). In this study, we considered boys with Vignos scores 1-6 as the ambulatory group (able to walk independently, with or without aids) and boys with Vignos scores 7-10 as wheel-chair-dependent. Boys with a Vignos score of 7 are able to walk small distance with aids and support, however in practice, they use a wheelchair most of the time. For this reason we included them in the non-ambulatory group. The Brooke scale classifies the arm function ranging from the ability to raise the arms above the head (0) to not being able to raise the hands to the mouth (6). We considered Brooke 1-3 as good arm ability (3 means being able to raise a glass of water to their mouth) and Brooke 4-6 as decreased arm function (4 means being able to raise hands, but not a glass of water, to the mouth).

The MFM measures the functional motor abilities of both ambulant and wheelchair-dependent patients with a neuromuscular disorder.[21] The 32 items of the MFM are classified in three dimensions: (1) standing and transfers, consisting of 13 items; (2) axial and proximal motor function, consisting of 12 items; and (3) distal motor function, consisting of 7 items. Each item is scored on a scale from 0 (does not initiate movement) to 3 (completes the item with a standard pattern). We calculated a percentage with a higher score indicating a better motor function.

The Abilhand questionnaire was used to measure the capacity to manage daily activities that require the use of the upper limbs. The questionnaire consists of 21 items which describe various activities (such as brushing teeth). The boys reported each activity "easy" (score 3), "difficult" (score 2) or "impossible" (score 1).[22] For boys who had significant problems with moving their arms, we used the original, extended (adult) version of the Abilhand consisting of 56 items.[23]

We used two domains of the PEDI structured interview[24] to assess the general functional status of the boys: (1) "selfcare", consisting of 73 items and (2) "ambulatory status" consisting of 64 items. The ambulatory status was not assessed in boys who had already been wheelchair-dependent for several years and had decreased arm function. Every item scored 1 point when the activity could be performed and 0 if the patient was unable to perform the activity.

Statistical analysis

Statistical analyses were conducted using SPSS version 16.0 for Windows (SPSS Inc., Chicago, Illinois, USA). We used the KS-52 syntax, based on the Rasch model to convert the raw data into *t*-values to describe both self-reported and proxy-reported HRQoL in comparison to the healthy population. Paired t-tests were used to measure the agreement between boys with DMD and their parents.

Correlations between the HRQoL using the raw data of the KS-52 and the different ICF domains were analyzed with two tailed, non-parametric Spearman's correlation coefficient (ρ). Correlation coefficients were interpreted as follows: 0 to 0.25: little to any correlation, 0.26 to 0.49: low correlation, 0.50 to 0.69: moderate correlation, 0.70 to 0.89: high correlation and correlations ≥0.90 indicated a very high correlation.[25] Because of multiple comparisons we chose a *p*-value of <0.01 as statistically significant.

Results

Forty boys with DMD, aged 8-20 years, participated in the study. Their characteristics are summarized in table 1. Nineteen boys were still ambulatory, while twenty-one boys were wheelchair-dependent. In the non-ambulatory group seven boys had a decreased arm function (Brooke ≥4), while 14 had a relative good arm ability (Brooke ≤3).

Table 1 Demographics and clinical data of the participants

	Total (n=40)	Ambulant (n=19)	Wheelchair-dependent (n=21)	
			Non-ambulant, relatively good arm abilities (n=14)	Non-ambulant, decreased arm abilities (n=7)
Demographics				
Age (years), mean (SD)	11.5 (3.4) (n=40)	9.1 (1.8) (n=19)	12.3 (2.3) (=14)	16.3 (2.6) (n=7)
Body structures				
MRC, median (range), left/right				
- Hip extensors	2.0 (1;4) (n=27)/	2.0 (1;4) (n=16)/	2.0 (1;3) (n=11)	NA
	2.0 (1;5) (n=27)	2.0 (2;4) (n=16)	2.0 (1;3) (n=11)	NA
- Knee extensors	3.0 (1;5) (n=28)/	4.0 (2;5) (n=16)/	2.0 (1;3) (n=11)	NA
	3.0 (1;5) (n=28)	4.0 (2;5) (n=16)	2.0 (1;3) (n=11)	NA
- Shoulder abductors	3.0 (1;4) (n=27)/	4.0 (2;4) (n=15)/	3.0 (2;4) (n=11)	NA
	4.0 (1;4) (n=27)	4.0 (2;4) (n=15)	3.0 (2;4) (n=11)	NA
- Elbow extensors	3.0 (2;5) (n=28)/	4.0 (3;5) (n=16)/	3.0 (2;4) (n=11)	NA
	3.0 (3;5) (n=28)	4.0 (3;5) (n=16)	3.0 (3;4) (n=11)	NA
A6MCT				
- Legs	423.0 (143.8) (n=20)	462.1 (153.0) (n=13)	350.3 (95.8) (n=7)	NA
- Arms	385.9 (111.7) (n=24)	388.6 (106.2) (n=13)	382.6 (123.1) (n=11)	NA
Activities and participation				
Vignos, median (range)	7.0 (2;9) (n=40)	2.0 (2;5) (n=19)	9.0 (6;9) (n=14)	9.0 (9;9) (n=7)
Brooke, median (range)	1.0 (1;5) (n=40)	1.0 (1;2) (n=19)	1.5 (1;3) (n-=14)	5.0 (4;5) (n=7)
MFM %, mean (SD)	68.5 (14.6) (n=30)			
Abilhand (sumscore), mean (SD)				
- Kids	51.9 (9.3) (n=21)	53.8 (7.7) (n=13)	52.3 (5.8) (n=7)	24 (n=1)
- Regular	107.9 (27.4) (n=9)	NA	114.0 (14.2) (n=3)	104.8 (33.0) (n=6)
PEDI (raw score), mean (SD)				
- Selfcare	41.7 (18.6) (n=40)	49.9 (14.3) (n=19)	42.0 (17.8) (n=14)	19.0 (11.1) (n=7)
- Ambulatory status	38.8 (17.1) (n=33)	47.7 (14.3) (n=19)	28.8 (11.1) (n=12)	14.0 (19.8) (n=2)

MRC, Medical Research Council Scale; NA, Not applicable; A6MCT, Assisted Six-Minute Cycling Test; MFM, Motor Fucntion Measure; PEDI, Dutch Pediatric Evaluation of Disability Inventory

The response rate of the KS-52 was satisfactory (>75%). Only the response rate on the financial domain was below 50% (19 out of 40), and this domain was therefore excluded from further analysis. Two proxy-reports were missing because of a language barrier.

Subscales of HRQoL in boys with DMD compared with their age-matched controls

Only the physical domain of the KS-52 was lower in the total group (mean = 40, SD = 6) than the reference group (mean = 50, SD = 5). In the ambulatory group the mean on the physical domain was 43 (SD = 6), in the non-ambulatory group with a relatively good arm function the mean on the physical domain was 36 (SD = 6) and in the group with decreased arm function 38 (SD = 2). This means that the boys felt less energetic and experienced to perform less physical activities than their age-matched controls and the physical domain seem to be lower in the non-ambulatory group compared to the ambulatory group. There were no differences between the self-reports of the boys with DMD and their age-matched controls in any of the other domains.

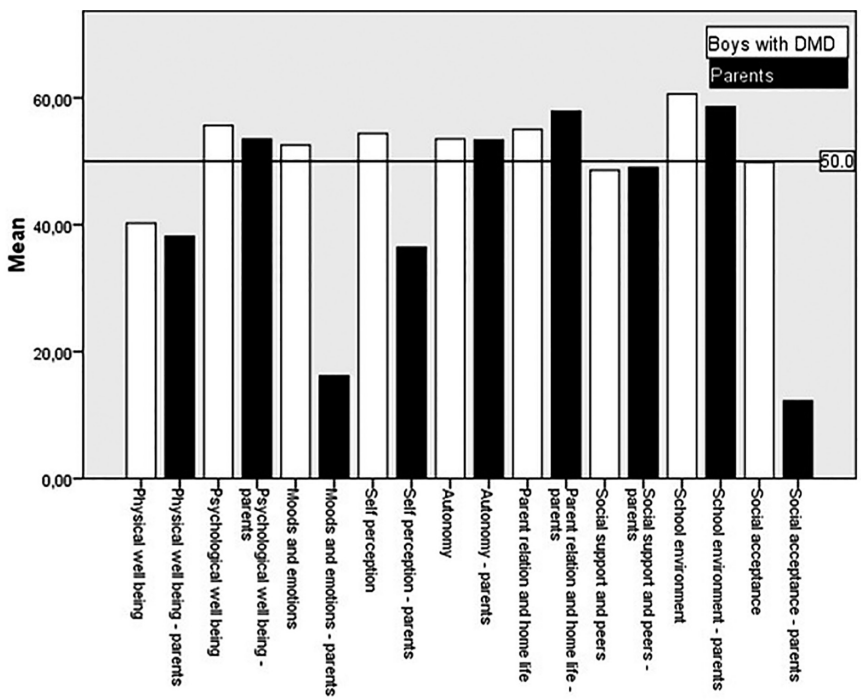

Figure 1 Subscales of HRQoL in the total group.

Comparison of the subscales of HRQoL in boys with DMD to their parents

Figure 1-4 show the comparisons between boys and parents on the different subscales of the KS-52 for the total group of boys with DMD (Figure 1) and the three subgroups (Figure 2-4). There was a good agreement between the boys with DMD and their parents on most of the domains of the KS-52, except for the domains "moods and emotions", "self perception" and "social acceptance". The parental perception of the HRQoL was significantly lower on these domains than the perception of the boys with DMD, respectively $t = 10.5$ ($p<0.00$), $t = 8.9$ ($p<0.00$), $t = 11.0$ ($p<0.00$) in the total group.

Correlations between the subscales of HRQoL and the ICF dimensions

Table 2 provides an overview of all correlations between the KS-52 domains and ICF-dimensions in the total group of boys with DMD. A moderate negative correlation between the domain "parent relation and home life" and the A6MCT of the arms ($\rho = -0.53$, $p = 0.01$), which means that boys with DMD perceive their relation with their parents better when they have decreased endurance in their arms.

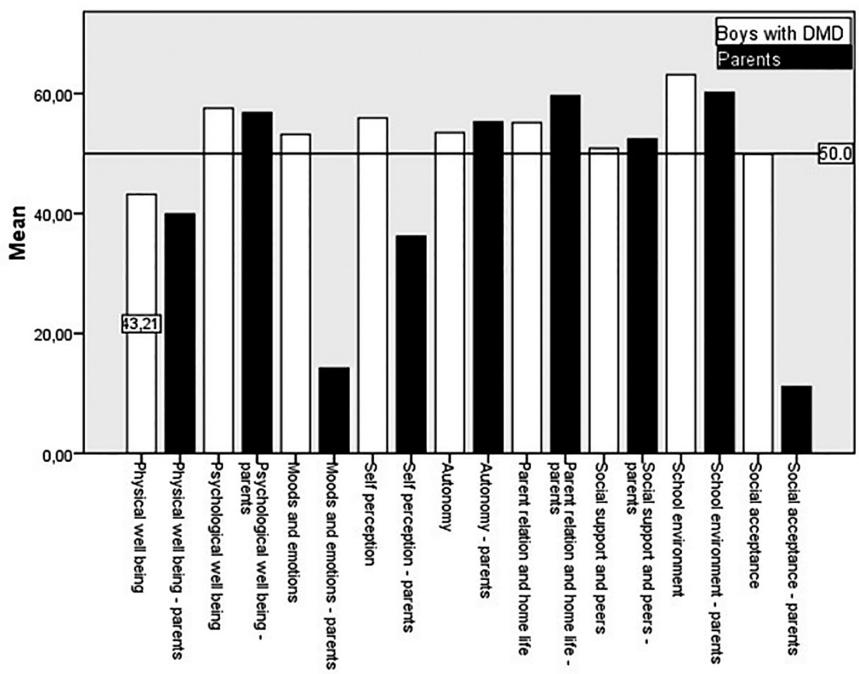

Figure 2 Subscales of HRQoL in the ambulatory group.

No other correlations were found between body functions and the KS-52 domains in all groups

Two significant correlations were found on activity level in the total group. First, a low negative correlation exists between the "physical domain" and the Vignos scale in the total group (ρ = -0.45, p = 0.01), which indicates that if the ambulation decreases, the perceived physical abilities decrease (as a higher vignos scale is associated with a lower ambulatory score). Second, we found a low positive correlation (ρ = 0.45, p = 0.01) between the "physical domain" and the PEDI "selfcare" domain, which means when boys with DMD are less able to take care of themselves independently, they perceive their physical abilities lower. The KS-52 was not correlated to the Brooke scale, the MFM and the Abilhand in the total group and ambulatory group.

In the non-ambulatory group we found a moderate negative correlation between the domain "parent relation and home life" of the KS-52 and dimension 1 of the MFM (standing position and transfers) (ρ = -0.603, p = 0.01). Furthermore, a moderate negative correlation was found between the domain "Autonomy" of the KS-52 and dimension 3 of the MFM (distal motor capacity) in the non-ambulatory group (ρ = -0.639, p = 0.01).

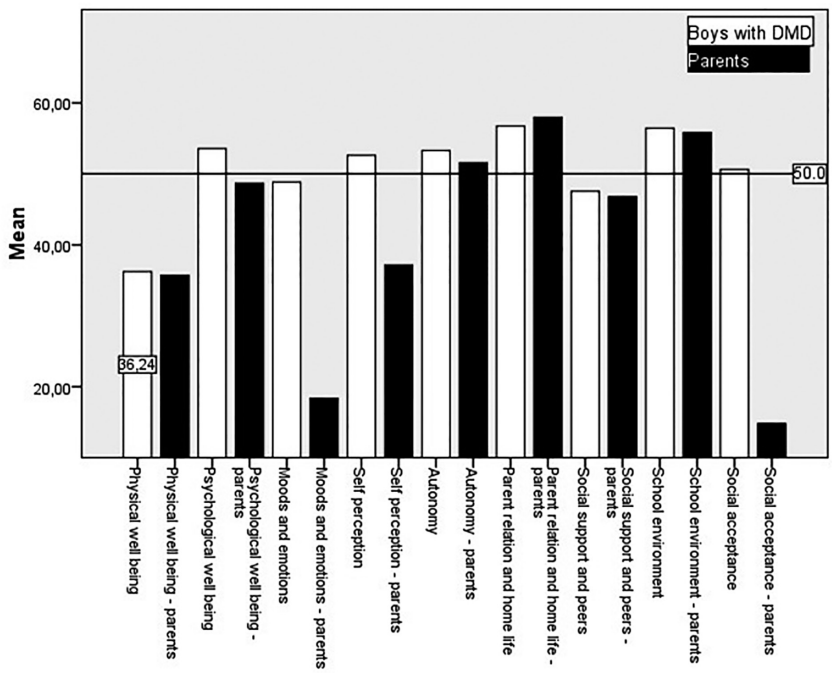

Figure 3 Subscales of HRQoL in the non-ambulatory group with good arm abilities.

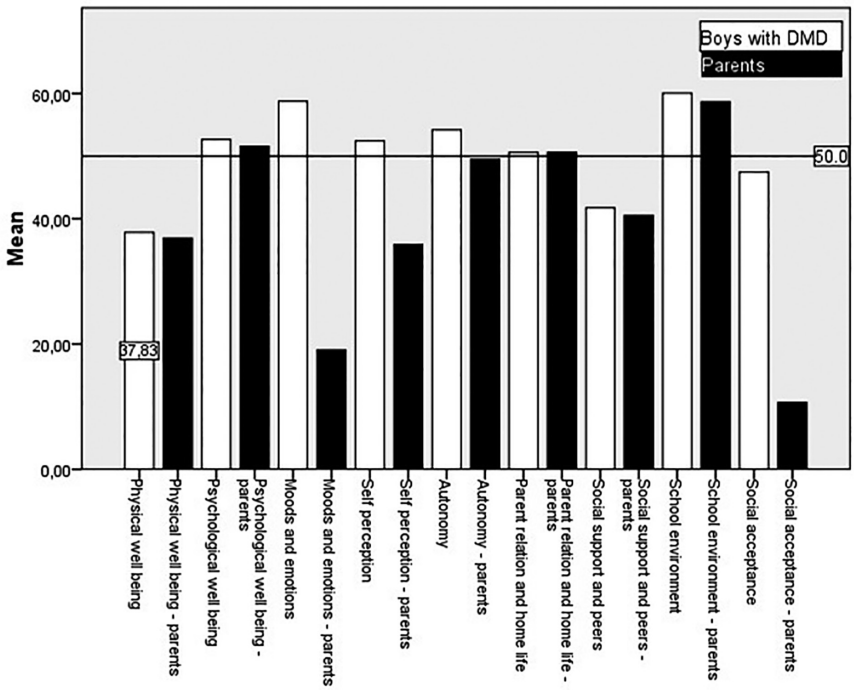

Figure 4 Subscales of HRQoL in the non-ambulatory group with decreased arm abilities.

Table 2 Subject characteristics and test results of healthy boys and boys with Duchenne muscular dystrophy

		Physical well being	Psychological bell being	Moods and motions	Self-perception
Body structures					
MRC left/ right					
Hip extensors		0.15(0.51) / 0.22 (0.33) n=21	0.26 (0.23)/ 0.10 (0.65) n=23	0.15 (0.51)/ 0.19 (0.40) n=22	0.11 (0.63)/ 0.10 (0.64) n=23
Knee extensors		0.45 (0.03)/ 0.38 (0.08) n=22	0.28 (0.18)/ 0.18 (0.40) n=24	0.06 (0.78)/ 0.32 (0.89) n=23	0.12 (0.59)/ 0.08 (0.72) n=24
Shoulder abductors		0.03 (0.90)/ -0.07 (0.77) n=22	0.01 (0.97)/ -0.10 (0.66) n=24	-0.12(0.60)/ -0.14 (0.54) n=23	-0.21(0.33)/ -0.26(0.21) n=24
Elbow extensors		0.45 (0.03)/ 0.38 (0.08) n=22	0.12 (0.58)/ 0.12 (0.56) n=24	0.07 (0.75)/ 0.13 (0.55) n=23	-0.06(0.78)/ 0.06(0.78) n=24
A6MCT	Legs	0.49 (0.06) n=16	0.89 (0.73) n=18	0.12 (0.65) n=17	-0.05 (0.84) n=18
	Arms	0.15 (0.53) n=19	-0.32 (0.16) n=21	0.03 (0.91) n=20	-0.34 (0.13) n=21
Activities and participation					
Vignos		**-0.45 (0.01) n=33**	-0.09 (0.60) n=33	-0.18 (0.32) n=33	-0.03 (0.86) n=33
Brooke		-0.34 (0.06) n=31	0.04 (0.84) n=33	0.21 (0.26) n=32	0.10 (0.57) n=33
MFM (total)		0.24 (0.28) n=22	-0.34 (0.87) n=24	-0.18 (0.42) n=23	0.00 (0.10) n=24
Abilhand	Kids	0.28 (0.25) n=19	-0.19 (0.44) n=20	0.11 (0.66) n=20	-0.31 (0.19) n=20
	Regular	0.52 (0.19) n=8	0.33 (0.43) n=8	0.11 (0.80) n=8	-0.22 (0.60) n=8
PEDI	Selfcare	**0.45 (0.01) n=31**	-0.04 (0.82) n=33	-0.04(0.84) n=32	-0.12 (0.52) n=33
	Ambulation	0.47 (0.02) n=25	0.15 (0.46) n=27	0.00 (0.99) n=26	0.08 (0.70) n=27

MRC, Medical Research Council Scale; NA, Not applicable; A6MCT, Assisted Six-Minute Cycling Test; MFM, Motor Fucntion Measure; PEDI, Dutch Pediatric Evaluation of Disability Inventory

Autonomy	Parent relation and home life	Social support and peers	School environment	Social acceptance
0.18 (0.42)/ -0.16 (0.49) n=22	0.01 (0.97)/ -0.14(0.52) n=23	0.28 (0.19)/ 0.04 (0.86) n=23	0.33 (0.13)/ 0.15 (0.52) n=22	-0.11(0.63)/ 0.31 (0.18) n=21
0.04 (0.88)/ -0.16 (0.46) n=23	-0.43 (0.84)/ 0.27 (0.21) n=24	-0.09(0.69)/ -0.08(0.70) n=24	0.20 (0.35)/ 0.11 (0.61) n=23	0.10 (0.65)/ 0.12 (0.61) n=22
-0.72(0.4)/ -0.32 (0.14) n=23	-0.33(0.12)/ -0.38 (0.07) n=24	-0.35(0.10)/ -0.28(0.19) n=24	-0.04(0.87)/ -0.13(0.55) n=23	-0.18(0.41)/ -0.06 (0.79) n=22
-0.17(0.43)/ -0.16(0.46) n=23	-0.35(0.09)/ -0.22(0.30) n=24	-0.15(0.48)/ -0.15(0.47) n=24	0.01(0.98)/ 0.01(0.95) n=34	0.18 (0.42)/ 0.08(0.73) n=22
0.07 (0.79) n=17 -0.43 (0.06) n=20	-0.14 (0.59) n=18 **-0.53 (0.01)** **n=21**	-0.17 (0.07) n=18 -0.18 (0.44) n=21	0.08 (0.77) n=18 -0.21 (0.38) n=20	0.07 (0.78) n=17 -0.13 (0.96) n=19
-0.09 (0.62) n=32	0.02 (0.90) n=33	-0.23 (0.19) n=33	-0.25 (0.17) n=31	-0.05 (0.80) n=31
0.14 (0.46) n=32	0.23 (0.20) n=33	-0.23 (0.21) n=33	-0.13 (0.50) n=32	0.04 (0.84) n=31
-0.29 (0.18) n=23	-0.37 (0.08) n=24	-0.33 (0.11) n=24	0.06 (0.78) n=23	-0.07 (0.76) n=22
-0.24 (0.32) n=20 0.28 (0.51) n=8	-0.26 (0.28) n=20 0.09 (0.84) n=8	-0.24 (0.31) n=20 0.42 (0.30) n=8	-0.10 (0.67) n=19 0.63 (0.10) n=8	0.09 (0.74) n=18 -0.20 (0.63) n=8
-0.18 (0.92) n=32 0.03 (0.89) n=26	-0.04 (0.83) n=33 -0.10 (0.64) n=27	0.27 (0.13) n=33 0.05 (0.79) n=27	0.25 (0.16) n=32 0.22 (0.28) n=26	0.02(0.94) n=31 0.16 (0.44) n=25

Discussion

The first finding we want to emphasize is that except for the "physical domain", the HRQoL is similar to their healthy peers and is not influenced by disease progression in boys with DMD in contrast to previous studies[8-10,15] were the HRQoL was lower in comparison to healthy peers. We presume that part of the differences is explained by the use of child-reports and proxy-reports in our study and our large control group of the KS-52 (n=22.827). Besides, the PedsQL used by Davis and Bendixen[8,15] has limited domains and is not designed to a full range of functioning like the KS-52.

This finding means that boys with DMD value their life positively, despite their limitations and disease progression. This forms a solid psychological basis in a continued search for life-lengthening therapies for boys with DMD and implies that when new therapies are applied, the HRQoL is not likely to improve (with the exception of physical domain), because it is similar to healthy peers at the start of a therapy. HRQoL assessment is a suitable instrument to evaluate whether or not a therapy has a negative impact on HRQoL. For example, too high expectations of a therapy might negatively influence HRQoL. Of course, it is a challenge to improve the physical domain.

The second finding we want to emphasize is the difference in perceived HRQoL of boys with DMD and their parents on the domains "mood and emotions", "self perception" and "social acceptance". The parents grossly underestimate the perception of their sons' happiness, the perception of their looks and the perceived support from their social network.

We expected such an underestimation based on previous studies[8,11], which found a poor to moderate agreement on all PedsQL domains, were the agreement on the social domain was poorest. In our study, we were able to further specify social sub domains to increase the insight on which level the agreement is lowest.

We have two hypotheses for the difference in perceived HRQoL between boys and their parents at these domains; (1) the parents reflect on their own frame of reference by interpreting the limitations of their sons or (2) the parents are more sensitive to negative comments of the environment. In literature no research is done on this topic to support our presumptions yet. Eiser[26] found some evidence that social support networks of families with a chronically sick child are smaller, denser and qualitatively lower from those of healthy families.

The poor agreement between the boys and their parents emphasizes the importance of self-report questionnaires when measuring HRQoL in boys with DMD. Besides, it is very important to inform parents about these differences. Hopefully, they will be comforted and involve their sons in therapy decisions.

The third and last finding we want to discuss is that we did not find any strong and recurrent correlations between the KS-52 domains and the outcome measures of the ICF model. This means that despite the decrease in activities, the boys with DMD stay content with their life. Here we will discuss the correlations we did find.

In the total group, the correlation between the "physical domain" of the KS-52 and the PEDI "selfcare" domain can be explained by decreased physical opportunities. We expected the low negative correlation between the Vignos scale and the "physical domain" , because the physical domain is the only HRQoL subscale which is influences by disease progression as mentioned before.

In the non-ambulatory subgroup we found two significant negative correlations on activity-level (MFM D1 and arm endurance) in relation to the KS-52 domain. This means that when the standing position and transfers of boys with DMD deteriorate or if the arm endurance decreases, they perceive a better understanding and more love from their parents and feel more happy at home. Biairdini[9] also found that ill children receive more attention and support from their family and suggested that this is because caregivers are more worried and distressed about their children's clinical conditions and they are more aware about the progressive nature. Besides, a moderate negative correlation between MFM D3 and the "autonomy" domain, which indicates that a decrease in distal motor function is associated with an increase in autonomy, meaning boys with DMD have enough time to themselves, to meet friends and to do the things they want in their free time. We think it is most likely that this association is not directly related to the disease progress in DMD but merely reflects growing up into adulthood.

Our study should be viewed in the light of its limitations. First, no specific outcome measure was used to assess participation-level in boys with DMD. At the start of the NUD study, no validated instrument was available to measure the participation-level separately in children, meanwhile such a questionnaire is developed and validated.[27] Bendixen[15] used a participation measurement in comparison with the HRQoL in boys with DMD. They emphasize further research is necessary as participation tends to decrease with age in boys with DMD. Their cohorts were divided based on age, while only three of the fifty participants were in the non-ambulatory phase. We recommend additional research including participation outcome measurements, based on life limiting events. Second, our study has a relatively small sample size, especially for analyzing subgroups. In a larger sample we expect significant positive correlations between activity-level and HRQoL domains could occur. Third, the KS-52 is not a disease specific questionnaire. As a result, one of the five items in the physical domain ("were you able to run well?") was not appropriate for all boys. Then again, the differences between the boys with DMD and the healthy peers and between the different ambulation groups may become even bigger when using a disease specific instrument. The final limitation of this study is that the response on the financial domain of the KS-52 was very low. An explanation could be that the teenage boys with DMD are not usually responsible for their own financial situation.

To conclude, boys with DMD have a stable HRQoL despite disease progression except for the physical domain. Information about the perceived HRQoL of boys with DMD to the parents is crucial as they tend to underestimate the HRQoL of their sons.

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Author Contributions

SLSHvO analyzed the data and wrote the first draft of the manuscript. MJ cared for patients, collected data, and made significant contributions to the manuscript. NvA and IJMdG were mentors who contributed equally to this work.

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Ethical Approval

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References

1. Hayley SM, Coster WI, Kao YC et al. Lessons from Use of the Pediatric Evaluation of disability Inventory. Where do we go from here? *Pediatric physical therapy* 2010;22:69-75.
2. Jansen M, de Groot IJ, van Alfen N, Geurts ACH. Physical training in boys with Duchenne Muscular Dystrophy: the protocol of the No Use is Disuse study. *BMC Pediatrics* 2010;10:55.
3. Kohler M, Clarenbach CF, Böni L et al. Quality of Life, Physical disability, and respiratory impairement in Duchenne Muscular Dystrophy. *Am J Respir Crit Care Med* 2005;172:1032-1036.
4. Eiser C, Morse R. Quality-of-Life measures in chronic diseases of childhood. *Health Technol Assess* 2001;5:1-157.
5. Arpinelli F, Bamfi F. The FDA guidance for industry on PROs: the point of view of a pharmaceutical company. *Health and Quality of Life Outcomes* 2006; 4:85.
6. U.S. Department of Health and Human Services FDA Center for Drug Evaluation and Research, U.S. Department of Health and Human Services FDA Center for Biologics Evaluation and Research, U.S. Department of Health and Human Services FDA Center for Devices and Radiological Health. Guidance for industry: patient-reported outcome measures: use in medical product development to support labeling claims: draft guidance. *Health and Quality of Life Outcomes* 2006; 4:79.
7. Grootenhuis MA, De Boone J, Van Der Kooij AJ. Living with muscular dystrophy: HRQoL consequences for children and adults. *Health and Quality of Life Outcomes* 2007; 5:31.
8. Davis S, Hynan LS, Limbers CA et al. The PedsQL in pediatric patients with Duchenne Muscular Dystrophy: feasibility, reliability, and Validity of the Pediatric Quality of Life Inventory Neuromuscular Module and generic core scales. *J of Clinical neuromuscular disease* 2010; 11:3.
9. Baiardini I, Minetti C, Bonifacino S et al. Quality of Life in Duchenne Muscular Dystrophy: The Subjective Impact on Children and Parents. *J Child Neurol* 2011;26:707-713.
10. McDonald CM, McDonald DA, Bagley A et al. Relationship Between Clinical Outcome Measures and Parent Proxy Reports of Health-Related Quality of Life in Ambulatory Children With Duchenne Muscular Dystrophy. *J Child Neurol* 2010; 25:1130-1144.
11. Bray P, Bundy AC, Ryan MM et al. Health-related Quality of Life in Boys With Duchenne Muscular Dystrophy: Agreement Between Parents and Their Sons. *J Child Neurol* 2010; 25:1188-1194.
12. Post M, Noreau L. Quality of life after spinal cord injury. *J Neurol Phys Ther* 2005;29:139-146.
13. World Health Organization. *International Classification of Functioning, Disability and Health.* WHO 2010. Available at http://www.who.int/classifications/icf/en/. Accessed August 2012.
14. Schiariti V, Fayed N, Cieza A et al. Content comparison of health-related quality of life measures for cerebral palsy based on the International Classification of Functioning. *Disability and Rehabilitation* 2011;33:1330-1339.
15. Bendixen RM, Senesac C, Lott DJ, Vandenborne K. Participation and quality of life in children with Duchenne muscular dystrophy using the International Classification of Functioning, Disability, and Health. *Health and Quality of Life Outcomes* 2012;10-43.
16. Ravens-Sieberer U. *The KIDSCREEN Questionnaires, quality of life questionnaires for children and adolescents. Handbook.* Lengerich: Pabst Science Publishers; 2006.
17. Ravens-Sieberer U, Gosch A, Rajmil L et al. KIDSCREEN-52 quality-of-life measure for children and adolescents. *Expert Rev Pharmacoecon Outcomes Res* 2005;5:353-364.
18. Ravens-Sieberer U, Gosch A, Rajmil L et al. KIDSCREEN Group The KIDSCREEN-52 Quality of Life Measure for Children and Adolescents: Psychometric results from a Cross-Cultural Survey in 13 European Countries. *Value in Health* 2008;11:645-658.
19. Lue YL, Su CY, Yang RC et al. Development and validation of a muscular dystrophy-specific functional rating scale. *Clinical Rehabilitation* 2006;20:804-817.
20. Jansen M, De Jong M, Coes HM et al. The assisted 6-minute cycling test to assess endurance in children with a neuromuscular disorder. *Muscle and Nerve* 2012;46:520-530.
21. Berard C, Payan C, Hodgkinson I, Fermanian J. A Motor function measure for neuromuscular diseases. Construction and validation study. *Neuromuscul Disord* 2005; 15:463-470.
22. Arnould C, Penta M, Renders A, Thonnard JL. ABILHAND-Kids: a measure of manual ability in children with cerebral palsy. *Neurology* 2004;63:1045-1052.

23. Penta M, Thonnard JL, Tesio L. ABILHAND: a Rasch-built measure of manual ability. *Arch Phys Med Rehabil* 1998;79:1038-1042.
24. Custers JW, Wassenberg-Severijnen JE, Van der Net J et al. Dutch adaptation and content validity of the Pediatric Evaluation Disability Inventory. *Disability and Rehabilitation* 2002; 24:250-258.
25. Munro BH. Correlation. In: Munro BH, editors. *Statistical methods for health care research*. 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2005, p 239-258.
26. Eiser C. Psychological Effects of Chronic Disease. *J Child Psychol Psychiatry* 1990;31:85-89.
27. Law M, King G, Petrenchik T et al. The assessment of preschool children's participation: internal consistency and construct validity. *Phys Occup Ther Pediatr* 2012; 32:272-87.

Part 2

Physical training in Duchenne muscular dystrophy

5

Physical training in boys with Duchenne muscular dystrophy: the protocol of the No Use is Disuse study

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Abstract

Background: "Use it or lose it" is a well known saying which is applicable to boys with Duchenne muscular dystrophy (DMD). Besides the direct effects of the muscular dystrophy, the increasing effort to perform activities, the fear of falling and the use of personal aids indirectly impair leg and arm functions as a result of disuse. Physical training could oppose this secondary physical deterioration. The No Use is Disuse (NUD) study is the first study in human subjects with DMD that will examine whether a low-intensity physical training is beneficial in terms of preservation of muscle endurance and functional abilities. The study consists of two training intervention studies: study 1 "Dynamic leg and arm training for ambulant and recently wheelchair-dependent boys with DMD and, study 2 "Functional training with arm support for boys with DMD who have been confined to a wheelchair for several years". This paper describes the hypotheses and methods of the NUD study.

Methods: Study 1 is an explorative randomized controlled trial with multiple baseline measurements. Thirty boys with a DNA-established diagnosis of DMD will be included. The intervention consists of a six-months physical training during which boys train their legs and arms with active and/or assisted cycling training equipment. The primary study outcomes are muscle endurance and functional abilities, assessed with a Six-Minute Bicycle Test and the Motor Function Measure. Study 2 has a within-group repeated measurements design and will include ten boys with DMD who have already been confined to a wheelchair for several years. The six-months physical training program consists of 1) a computer-assisted training and 2) a functional training with an arm support. The primary study outcome is functional abilities of the upper extremity, assessed with the Action Research Arm Test.

Discussion: The NUD study will fill part of the gap in the current knowledge about the possible effects of training in boys with DMD and will increase insight into what type of exercise should be recommended to boys with DMD. The study will finish at the end of 2010 and results are expected in 2011. Trial registration The Netherlands National Trial Register1631.

Background

Duchenne muscular dystrophy (DMD) is an inherited X chromosome-linked recessive myopathy which affects approximately 1/4200 live-born boys.[1] DMD is characterized by a total, or near-total (<3%), absence of the cell membrane protein dystrophin. The absence of dystrophin results in a steady degradation of muscle fibers that causes progressive loss of muscle strength and functional abilities.[2,3] Boys with DMD are usually confined to a wheelchair at the age of ten years[4] and have a median life expectancy of thirty years with spinal surgery and assisted ventilation[5]. Although ongoing studies show promising therapies that target disease cause, there is still no curative (pharmaco)therapy available and, thus, treatment remains symptomatic. An important aim in the management of boys with DMD is to preserve functional abilities for as long as possible.[6] Delaying the loss of functional abilities is relevant for all activities in daily life and may optimize independence in boys with DMD.

The loss of functional abilities is primarily the result of a progressive decrease in muscle strength and muscle endurance during the course of the disease.[4,7] However, increasingly limited physical and social possibilities gradually cause a secondary reduction of physical activity. Indeed, the increasing amount of energy a certain activity costs, the increasing frequency of falling (with the need for help to stand up), and the developing fear of falling further reduce leg and arm activities, resulting in disuse of the musculoskeletal and cardio-respiratory systems.[8] The use of an electrical wheelchair limits arm functions (like reaching and lifting) even more, since a top blade and a central operating joy stick force boys to function within the confines of the wheelchair. As the increased sedentary lifestyle results in progressive disuse, secondary physical deterioration will occur in all boys with DMD. Disuse in DMD thus can be defined as the discrepancy between a boy's potential capacity and his actual performance. To underscore the importance of disuse, previous studies have shown that the presence of hip, knee and elbow flexion contractures is strongly related to the onset of wheelchair dependence.[4] Another example is that boys with DMD have a higher risk of bone fractures due to osteoporosis caused by unloading.[9] Fractures as a result of falling are followed by a loss of ambulation in 20-40 percent of the cases.[10,11] In these aspects, the well-known saying "use it or lose it" is certainly applicable to boys with DMD.

Physical training could oppose the secondary deterioration of muscle tissue and the loss of functional abilities as a result of disuse. However, the number of studies that examined the effects of training in DMD is limited, and only a few training studies are reported in human subjects with DMD. These studies focused on resisted exercises in ambulatory boys and concluded that sub-maximal resistance exercises had only limited positive effects on muscle strength and timed functional tests (such as the time it takes to walk 23

feet) but, importantly, they did not cause physical deterioration.[12,13,14] Recent studies in mdx mice (an animal model for DMD) concluded that voluntary wheel running (dynamic training) had positive effects on muscle strength[15] and fatigue resistance[15,16,17]. In addition, non-weight bearing low-intensity exercises (like swimming) had no detrimental effects in mdx mice.[18] However, extrapolating data from animal studies to humans should only be done with great caution because of differences in phenotypic expression and biomechanical differences between humans subjects and animal models with muscular dystrophy.[19] Based on the currently available evidence, and clinical experience, international guidelines recommend ambulatory boys to perform voluntary (eventually mechanically-assisted) active exercises (such as swimming) and to avoid eccentric exercises. Non-ambulant boys are advised to perform mobilizing passive or actively-assisted mobilizing exercises to maintain postural symmetry and sitting comfort.[20]

The mechanisms by which training may oppose the deterioration of muscle tissue is unclear. Muscle fibres in DMD are abnormally vulnerable to contraction-induced injury due to the absence of mechanical reinforcement of the sarcolemmal membrane.[21] Therefore, eccentric exercises should be avoided. On the other hand, work-induced damage can enhance muscle regeneration and repair[23], and low-stress exercise may produce beneficial effects on myofiber contractility and energetic efficiency[21]. For example, low-intensity training decreased oxidative stress markers[24] and caused a shift from fast-twitch muscle fiber type 2 to slow-twitch muscle fiber type 1 in mdx mice.[16] Slow-twitch muscle fibers are more resistant to muscle fiber degeneration.[16,25] In addition, corticosteroids could support the beneficial effect of training in DMD, since steroids may prevent post-exercise deterioration of skeletal muscle.[22]

The currently recommended voluntary exercises to maintain comfort and symmetry[6] are widely used in daily practice and assume that low-intensity training is beneficial and not harmful for boys with DMD. However, these recommendations are mainly based on theory and there is a need for more research to justify these recommendations and to define the optimal exercise programs[26]. As we expect that a low-intensity training may slow-down the secondary decline of muscle endurance and functional abilities (Figure 1), we developed the *No Use is Disuse* (NUD) study protocol to answer the clinically relevant questions whether low-intensity physical training in boys with DMD is beneficial and does not cause further harm. The NUD study is the first to examine the effects of low-intensity physical training in human subject with DMD and will fill part of the gap in current knowledge about the possible effects of training in boys with DMD. The study consists of two training intervention studies: study 1 "Dynamic leg and arm training for ambulant and recently wheelchair- dependent boys with DMD and, study 2 "Functional training with arm support for boys with DMD who have been confined to a wheelchair already for several years". This paper describes the hypothesis and methods of the NUD study.

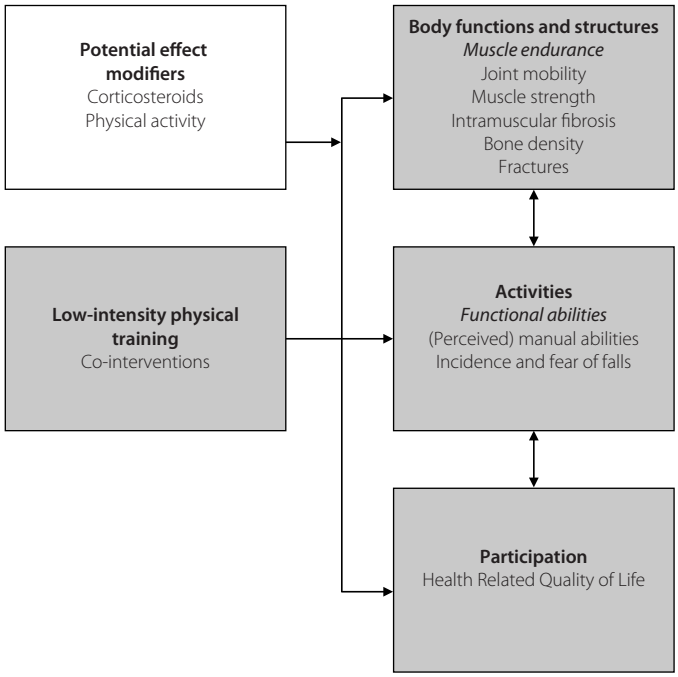

Figure 1 Hypotheses.

Methods

Study 1 Dynamic leg and arm training

Design

Study 1 is an explorative randomized controlled trial with multiple baseline measurements. Randomization is stratified according to the ambulatory status (ambulant / non-ambulant) of the boys and the boys are allocated to the intervention group or the control group in a 2:1 ratio. The intervention group receives dynamic exercise treatment, whereas the control group receives usual care (no specific intervention) during 24 weeks. After this period, the control group will also receive the physical training. Randomization is performed by an independent statistician. Despite randomization, differences in baseline characteristics between the intervention group and the control group may still be present due to the relatively small number of participants. For this reason, multiple baseline measurements are performed that will allow us to do within-subject analyses as well. Assessments are not blinded, because it was considered virtually impossible to prevent the boys from revealing their group allocation to the assessor.

Participants

Thirty ambulatory or recently wheelchair-dependent boys with a DNA-established diagnosis of DMD will be included (Figure 2). There are an estimated four hundred boys with DMD in the Netherlands. Inclusion and exclusion criteria are described in Table 1. Participants will be recruited from the Dutch Duchenne Parent Project (DPP) database. Members of the DPP will receive written information and a registration form. Additionally, we will place an advertisement on the website of the Vereniging Spierziekten Nederland (VSN), and rehabilitation physicians affiliated with the VSN will be asked to make potential participants aware of the NUD study. After registration, interested potential participants are visited by the primary investigator (MJ) at home for providing further information. Parents, and participants who are over 12 years of age, need to give written informed consent. The study protocol was approved by the Medical Ethics Committee Arnhem-Nijmegen, the Netherlands.

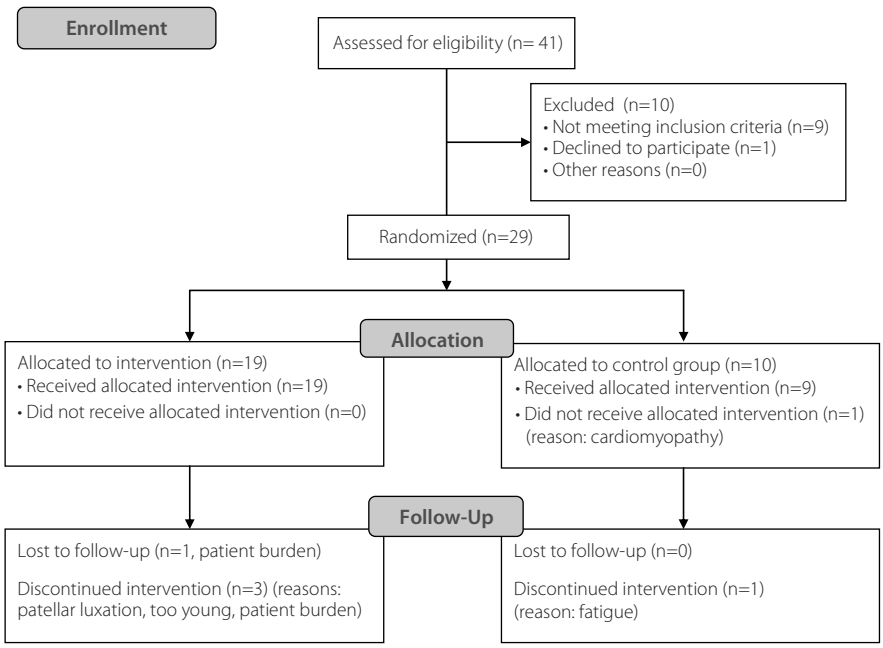

Figure 2 Progress study 1 Dynamic leg and arm training.

This flowchart is a preliminary version. Final numbers may change depending on eligibility as recruitment is not yet complete.

Table 1 Inclusion and exclusion criteria study 1

Inclusion criteria	Exclusion criteria
A DNA-established diagnosis of DMD	Other disabling diseases influencing mobility
Boys who are at the end of their ambulation phase, and: - need ≥5 sec. to get up from the floor or - are not able to get up from the floor or - are not able to bicycle without assistance - are dependent on a wheelchair to move over a long distance (>500m)	Boys with a clinical symptomatic cardiomyopathy Boys <6 years old
Boys who recently became wheelchair-dependent (approximately 1-2 years after they stopped walking), and: - are able to touch the top of their head with both hands without assistance or - are able to use a hand-operated wheelchair	

Intervention: dynamic leg and arm training

Boys train their legs and arms with bicycle training equipment (KPT Cycla, Kinetic, France). As this type of training equipment can be used actively or with electrical motor support, it allows a combination of active and passive bicycle training. Physical training is additional to any regular therapy or daily physical activity that boys already take part in, yet all possible co-interventions are registered.

Boys train at home or at school (depending on their preferences) during 30-min sessions (15 min leg and 15 min arm training), five days per week during 24 weeks. They are instructed to cycle with a continuous speed and stimulated to reach 700-1000 revolutions with both their legs and arms during each training session without getting too tired. Cycling characteristics are standardized (see Chapter 3, Figure 1) and crank-arm length is adjusted to the child's height (e.g. a short crank-arm length for small legs and arms). Boys are free to choose when they would like to train, their training sequence (first arms or legs) and whether they want a break between the two 15 min training sessions or not. However, these training agreements are decided beforehand and registered in a training contract to which they should adhere. Additionally, the boys are recommended to train while watching television to make the training more pleasurable.

Based on general training principles and based on the results from previous training studies in boys with DMD and in animal models of DMD, the training intensity is low to moderate. Intensity is determined by an adjusted and modified Six-Minute Bicycle ergometer Test (see study outcomes). Boys are encouraged to reach as many revolutions as possible during this sub-maximal test. The number of revolutions is an indication for training intensity (level of electrical assistance). After six minutes of rest, boys cycle for three more minutes at the indicated training intensity. During this period, they should be able to cycle with a continuous speed of 60 revolutions per minute (RPM) with a perceived level of exertion ranging from 'a little tired' to 'getting more tired', as assessed with the OMNI Scale of perceived exertion.[27] The primary investigator will check the training intensity once again during a 15 min training session within two weeks after the start of the intervention period. This control is carried out in a home setting, so that training posture can be checked as well. When boys are not able to bicycle at a continuous speed of approximately 60 RPM without having pain or maintaining the level of 'getting more tired', the training intensity will be lowered.

As described above, training intensity is based on the ability to bicycle at a continuous speed and perceived exertion. Intensity is, thus, not based on peak heart rate, which is common in the literature on physical exercise training. The reason for this is twofold. Firstly, boys with DMD have a higher resting heart rate (110±12 beats/min) compared to healthy controls (94±7beats/min).[28] Additionally, boys with DMD are often forced to terminate a bicycle ergometry test while their peak heart rate is only 120-130 beats/min, since the main limiting factor during ergometry seems to be not their oxygen transport but their 'peripheral' capacity (muscle endurance, anaerobic power and muscle strength).[7]

Boys are instructed to send their daily number of revolutions and their perceived levels of exertion to the primary investigator by a postal questionnaire once every two weeks, together with an overstrain questionnaire. Parents and their boys are informed by telephone when compliance is inadequate or when other problems (such as signs off overstrain: see adverse events) are identified and children are encouraged to resume the training or to adjust the training intensity.

Study outcomes

Study outcomes include measurements at the levels of body functions and structures, activities and participation as defined by the International Classification of Functioning, Disability and Health (ICF).[29] Environmental characteristics (school type and home status) are assessed at baseline. Demographic variables (age, weight and arm span), the use of medication (such as corticosteroids), any co-interventions and daily amount of physical activity are registered during each session.

The assessments of boys in the intervention group are conducted during the baseline period (T0: at baseline; T1: after 4 weeks; T2: after 8 weeks), the training period (T3: after 12 weeks; T4: after 24 weeks) and the follow-up (T5: 4 weeks after the end of the training; T6: 24 weeks after the end of the training). The assessments of boys in the control group are conducted during the baseline period (T0: at baseline; T1: after 4 weeks; T2: after 8 weeks), the control period (T3: after 12 weeks; T4: after 24 weeks), the subsequent training period (T5: after 12 weeks of training; T6: after 24 weeks of training) and during the follow-up (T7: 4 weeks after the end of the training). Assessments take place at the department of Rehabilitation of the Radboud University Nijmegen Medical Centre (T0, T2, T4, T6), but also at home (intervention group: T1, T3, T5; control group: T1, T3, T5, T7) to reduce the practical burden on the participants. All data are collected by the (trained) primary investigator (MJ). (Figure 3)

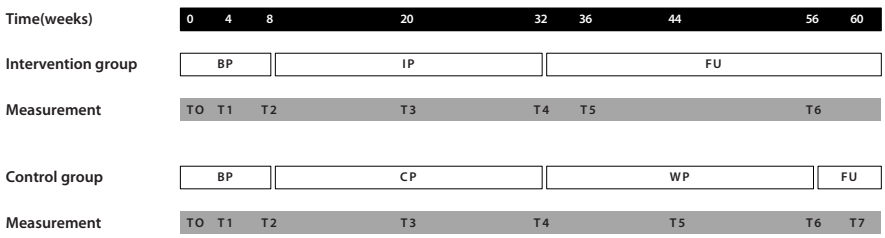

Figure 3 Measurements study 1.

BP, Baseline period; IP, Intervention period; FU, Follow-up; CP, Control period

5

Primary outcomes

Bicycle ergometry: Six-Minute Bicycle Test

Muscle endurance, in this study defined as 'the ability to sustain a power without the occurrence of peripheral fatigue', is assessed with an adjusted and modified bicycle ergometer test for both the lower and upper extremities. Bicycle ergometry has been used in many studies to examine the (an)aerobic performance in healthy children and children with disabilities, and several exercise protocols are available. However, these protocols are not feasible for boys with DMD, since large muscle groups are affected in DMD and initial load and subsequent increments of load are often too hard to sustain for these patients.[7,30] Muscle endurance is, therefore, examined with a recently developed motor- assisted (passive mode 1, no-load speed 7 RPM) Six-Minute Bicycle Test by which boys are instructed to cycle as fast as possible i.e. to make as many cycling revolutions as possible. Boys start with a test for their legs and, thereafter, perform the same (arm cranking) test with their arms.

The occurrence of muscle fatigue during bicycle ergometry is assessed objectively with bipolar surface electromyography (sEMG), since peripheral fatigue is reflected as an increase in the amplitude and a decrease in the median frequency of the sEMG signal.[31,32] These parameters were found to be responsive to changes in muscle endurance during an exercise program.[33,34] Electrodes are placed unilaterally (right leg, right arm) on the m. rectus femoris, m. vastus medialis, m. biceps femoris, m. tibialis anterior, m. biceps brachii, m. triceps brachii and m. deltoideus. Electrode placement procedures will follow the recommendations for Sensor and Sensor Placement Procedures for surface electromyography.[35] Data will be registered with an EFA system (Twente Medical Systems International). The occurrence of muscle fatigue is also assessed subjectively with the OMNI Scale for perceived exertion.[27] The OMNI Scale is reliable and valid over a wide range of dynamic exercise intensities in children.[27,36]

Motor Function Measure

The MFM is a recently developed instrument to assess motor function in ambulant and non-ambulant patients with neuromuscular diseases (NMD) aged 6-62 years.[37] The MFM has shown excellent internal consistency and good to excellent intra- and interrater reliability in French neuromuscular patients.[37] Vuillerot et al.[38] showed that the MFM is able to measure changes in motor function over time in boys with DMD. An ongoing study examines validity and applicability of the MFM in rehabilitation institutes in the Netherlands.

Secondary outcomes

Table 2 shows an overview of the secondary study outcomes and their psychometric properties.

Adverse events

All adverse events will be recorded. The study will be terminated prematurely for an individual participant when training is excessive and remains excessive after a reduction of the training intensity. Signs for overstrain are: excessive muscle pain during the training, prolonged post-exercise muscle pain, a severely uncomfortable feeling during or after the training and extreme (muscle) fatigue (OMNI scale > 6).

Sample size

The average rate of decline (mean and standard deviation (SD)) of muscle endurance and functional abilities in boys with DMD, as assessed with bicycle ergometry and the Motor Function Measure (MFM), is unknown. Therefore, no sample size can be calculated for these primary outcomes. However, it is anticipated that multiple baseline measurements will increase the chance of finding statistically significant changes in the selected outcome measures. For reasons of convenience, and because this RCT should be regarded as a first explorative study, we decided to include 20 boys in the intervention group and 10 boys in the control group.

Analysis

A two-way analysis of variance (ANOVA) will be used to examine the effects of dynamic leg and arm training on the primary outcomes. Secondary outcomes will be treated similarly or by non-parametric analyses dependent on the type of outcome (interval or ordinal). Data will be analyzed with SPSS version 16.0 and an alpha level of 0.05 will be used to decide on statistical significance.

Study 2 Functional training with arm support

Design

Study 2 has an observational one-group design using repeated measurements, because we anticipate that it will not be possible to recruit a sufficient number of boys for an RCT in the Netherlands. This problem is related to the fact that boys with DMD who have been confined to a wheelchair already for several years often experience many barriers, such as a lack of time due to therapy obligations and homework. It is also virtually impossible for them to do without their electric wheelchair for a couple of days to build up the required arm support for this study. In addition, the number of boys at this stage in the course of DMD is low (i.e. an estimated 120 boys in the Netherlands) and, it is expected that some of these boys already use an arm support, which is an exclusion criteria for participation.

Participants

Ten wheelchair-dependent boys with a DNA-established diagnosis of DMD will be recruited in the same way as the participants of study 1 (Figure 4). Inclusion and exclusion criteria are described in table 3. Participants will receive written information and will be visited by the primary investigator at home for providing further information. Both parents and participants will need to give written informed consent. The study protocol was approved by the Medical Ethics Committee Arnhem-Nijmegen, the Netherlands.

Intervention

The six-months physical training consists of 1) a computer-assisted training and 2) a functional training of the non-dominant arm and hand (i.e. the hand one does not use for writing) with a mechanical or an electrical arm support (Dynamic Arm Support Top/Help, Focal, Tilburg, the Netherlands) (Figure 5). Boys who are unable to touch their nose with a mechanical arm support (due to insufficient muscle strength) will receive an electrical arm support. The arm support transposes shoulder movements into movements of the forearm and hand, which increases functional abilities and joint range of motion in boys with DMD. During the first baseline measurement (T0), boys will try to fit the arm support under supervision of an experienced representative of Focal (the manufacturer of the TOP arm support) and the primary investigator. Training of the non-dominant arm is additional to any regular therapy. All co-interventions will be registered.

Table 2 Outcome measures and psychometric properties

Level	Study outcome	Measurement tool	Psychometric properties	Assessment
Body structures and functions	Muscle endurance	Six-Minute Bicycle Test*	Feasible for ambulant and non-ambulant boys with DMD (pilot study, unpublished data)	T0, T2, T5
	Joint mobility (PROM)	Goniometry (knee ext*, ankle dfl*, shoulder abd*†,elbow ext*†,wrist ext†, wrist radial and ulnar dev†)[54]	Standardized methods are feasible[55] and have good intra- and inter-rater reliability in DMD[57] Passive wrist radial deviation is correlated with functional hand activities[57] and lower extremity contractures are related to onset of wheelchair reliance in DMD[4]	All
	Muscle strength	Modified MRC (hip ext*, knee ext* ankle dfl*, shoulder abd*†, elbow ext*†, wrist ext†)[58]	Moderate to good intra- rater reliability[58] and acceptable inter- rater reliability in DMD after a training session[59] Muscles with MRC grade 4 or 5 are difficult to measure with MMT, but muscles with MRC grade ≤3 are more difficult to measure with HHD[4]	All
	Muscle atrophy, intra-muscular fibrosis and fatty infiltration	Quantitative skeletal muscle ultrasonography of the RF, TA, BB and FF (muscle thickness and echo intensity)[60]*†	Good inter- rater agreement in children[61] High predictive values to discriminate between children with and without a NMD[62]	T2, T5, T6/T7*
	Bone density	Dexascan (femur and lumbar spine)*	Changes in bone mineral density can be detected with confidence in healthy boys ≥10 years after 6 months and in younger boys after 12 months[63] ,but a change in body shape may influence scan results[64]	Conventional protocol for each boy
	Incidence of fractures	Semi- structured interview*		All
Activities	Functional abilities	Motor Function Measure (D1*, D2*,D3*†)[37]	Excellent internal consistency for the global scale and the subscales in NMD[37] Excellent to moderate intra- and inter-rater reliability in NMD[37] Good face validity, convergent validity and discriminant validity in NMD[37] Sufficiently sensitive to detect changes in the total score in DMD[38] Total score predicts loss of ambulation in DMD[38]	All

	Upper limb function	Action Research Arm Test[40,41 †]	Excellent intra-rater, inter-rater and test- retest reliability in stroke patients[40,41] Highly correlated with the Fugl-Meyer score and Functional Independence Measure in stroke patients[42] Suitable to detect changes over time in stroke patients[42]	All
	Functional abilities (grading)	Vignos* and Brooke Scale[55*†]	Good inter-rater and intra-rater reliability[56] and correlated with timed tests[46,65,66] in DMD	All
	Functional mobility	Functional Mobility Scale[67] *	A clinically feasible, valid and reliable tool in CP[67,68]	All
	Functional abilities (timed tests)	Timed and graded functional tests (and total GSGC score)[69]: walk 10 meters, climb 3 stairs, rise from the floor and rise from a chair*	Good to excellent intra- and inter-rater reliability in DMD[56,70] Sensitive to change in DMD: a small reduction in muscle force was accompanied by a large increase in time it takes to complete functional tests[71]	All (gait, stairs and chair only in the hospital)
	Finger dexterity	Nine- hole Peg Test[72*†]	Moderately high test-retest reliability, high inter-rater agreement and adequate concurrent validity in school-age children[73]	All
	Hand function	Jebsen-Taylor Hand Function Test[74†]	Good test-retest reliability in DMD[57] Strongly correlated with muscle strength of the wrist extensors[57], radial deviation range of motion[57] and the Brooke scale[46] in DMD	T2, T4, T5
	Functional status	PEDI (selfcare*† and mobility*)[75,76]	Good inter- rater and test-retest reliability[77], content validity[76] and discriminative validity[78] in children with various diagnosis Feasible for evaluative purposes in CP[78,79]	T0, T2, T4, T6/T7*
	Perceived manual abilities	Abilhand[80†] Abilhand-kids[81*]	The Rasch-derived Abilhand is moderately related to grip and key pinch strength, has good test-retest reliability and may be sensitive to change in stroke patients[82] The Abilhand-kids has good test- retest reliability and a higher independence in gross motor function is associated with a higher manual ability in CP[81]	T0, T2, T4, T6/T7*

Table 2 Continued

Level	Study outcome	Measurement tool	Psychometric properties	Assessment
	Quality of upper-limb motor function	Melbourne Assessment of Unilateral Upper Limb Function[83] (item 1,2,3,10,11 and16) extended with an upper limb motion analysis (Vicon Motion Systems) with 8 cameras[†]	The Melbourne Assessment has moderate to high intra- and inter- rater reliability[84] and excellent construct validity in CP[85] A motion capture analysis system can measure task performance with an upper-limb orthosis[45], but soft tissue artefacts may negatively influence accuracy[48]	T2, T4
	Incidence and fear of falls	Semi-structured interview*		All
Participation	HRQoL	KIDSCREEN-52 (child- and parental questionnaire)[86*†]	Acceptable levels of reliability and validity in children and adolescents[87] Children's most important in their lives generally map well to the items in KIDSCREEN[88]	T0, T2, T4, T6/T7*
Demographic variables	Weight and height	Body weight (kg)*[†], standing height* (cm) and arm-span*[†] (cm)		T0*, T2, T4, Y6/T7*
Co-factors	Co-interventions	Semi-structured interview*[†]		All
	Physical activity	Semi-structured interview (according to the PAQ-C[89] and the 60-min MVPA measure[90])*[†]		All

*study 1 'Dynamic leg and arm training'
[†]study 2 'Functional training with arm support'
DMD, Duchenne Muscular Dystrophy; PROM, Passive Range of Motion; ext, extension; dfl, dorsal flexion; abd, abduction; dev, deviation; MRC, Medical Research Council Scale; RF, m. Rectus Femoris; TA, m. Tibialis Anterior; BB, m. Biceps Brachii; FF, forearm flexors; NMD, Neuromuscular Disease; GSGC, Gait, Stairs, Gowers, Chair; PEDI, Pediatric Evaluation of Disability Inventory; CP, cerebral palsy; HRQoL, Health-Related Quality of Life; PAQ-C, Physical Activity Questionnaire for older children; MVPA, Moderate-to-Vigorous Physical Activity

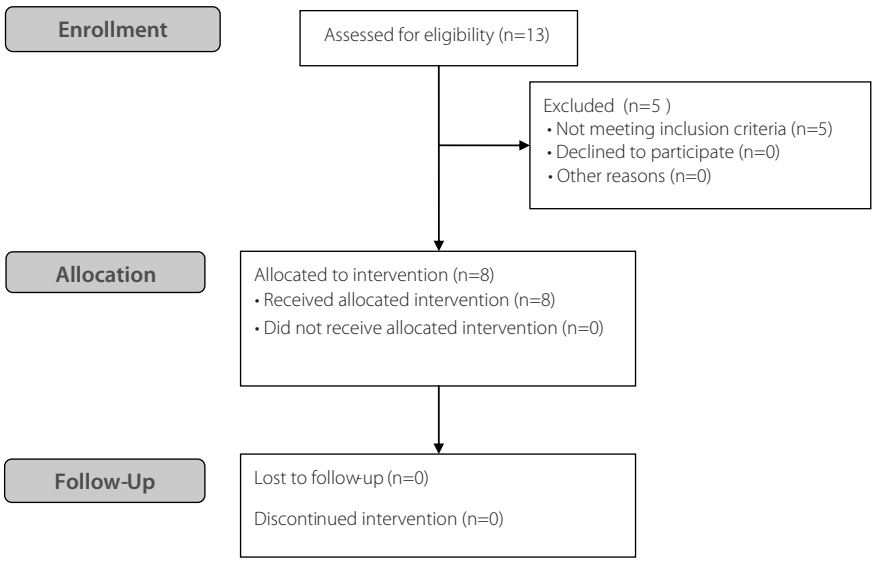

Figure 4 Progress study 2 Functional training with arm support.

This flowchart is a preliminary version. Final numbers may change depending on eligibility as recruitment is not yet complete.

Table 3 Inclusion and exclusion criteria study 2

Inclusion criteria	Exclusion criteria
A DNA-established diagnosis of DMD	Other disabling diseases influencing mobility
Boys who have been wheelchair-dependent for a few years (approximately 2-5 years after they stopped walking)	Boys who are able to stand
	Boys >20 years old
Boys who have problems with reaching and lifting movements with their arms, and: -are unable to touch the top of their head (at least with one hand) -are still able to use their hands for some daily activities	Boys who already use an arm support

Figure 5 Image of a Top/Help Dynamic Arm Support (DAS).
Courtesy of Focal Meditech BV, Tilburg, the Netherlands.

Computer-assisted training

Boys play "Furballhunt" (developed by Roessingh Research and Development B.V. (M.A. Jannink), Enschede, the Netherlands) to practice targeted forward and sideward reaching movements (ipsi- and contra-lateral) as well as lifting movements during five days per week. Furballhunt is a computer game that was originally developed to improve arm function in children with cerebral palsy using a virtual reality environment. Furballhunt is based on motion capture technology and uses a webcam (in this study: Logitech QuickCam E3500) to detect gross shoulder and elbow movements. Birds ("Furballs") fly from their bird- house to a tree branch while the boy holds his hand in front of his navel. Boys have to touch the Furball when it sits down on a tree branch. The faster boys chase away Furballs, the higher their score. A virtual reality computer game is considered to be a motivational tool for training in children.[39]

Boys play five games of Furballhunt (30 Furballs per game, 30 sec of rest between games) per day at home. The number of tree branches (3), game speed (5/10), the number of Furballs (30) and the sequence of games are standardized. Training intensity is low to moderate, which means that boys are allowed to perceive their exertion as 'getting a little tired' or 'getting more tired' as assessed with the OMNI Scale.[27] Intensity is adjusted to the physical abilities of the boy by varying the position of the tree branches, which represent the targets to move to. Boys are instructed to move their arm over the full range of motion, which may cause a feeling of stretch but not pain.

Boys report their training frequency in a diary and complete an overstrain questionnaire once every two weeks. Parents and their boys are informed by telephone when compliance is inadequate or when other problems (such as signs of overstrain: see adverse events) are identified. Boys are encouraged to resume the training and to adjust the training intensity, when appropriate. The date, training time and reaction times are saved on the computer, so that compliance and performance can be checked afterwards by the primary investigator.

Functional training

Boys should eat at least one meal with the arm support twice every week. In addition, they are instructed "to use the arm support as much as possible every day". Boys keep a written record of all activities they perform with the arm support in a three-day diary once every two weeks (two weekdays, one weekend day).

Study outcomes

Study outcomes include measurements at the levels of body functions and structures, activities and participation of the ICF.[29] Environmental characteristics (school type and home status) are assessed at baseline. Demographic variables (age, weight, arm span), the use of medication (such as corticosteroids) and any co-interventions are registered during each session.

After a two-month period for baseline measurements (T0: at baseline; T1: after 4 weeks; T2: after eight weeks) to obtain information about the stability of the course of DMD, training with the arm support takes place for six months. Assessments take place after 12 weeks (T3) and 24 weeks (T4) training. Finally, one extra measurement will be done after 12 more weeks (T5) to evaluate to what extent the possible effects of training have lasted. Data is collected by the primary investigator (MJ) at the department of Rehabilitation of the Radboud University Nijmegen Medical Centre (T2, T4), but also at home (T0, T1, T3, T5) to reduce the practical burden on the participants. (Figure 6)

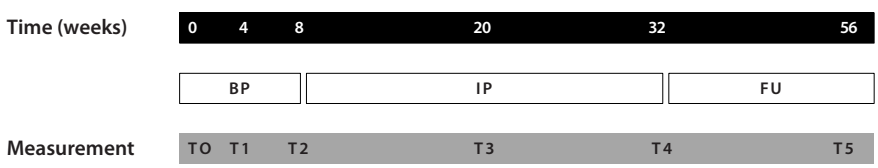

Figure 6 Measurements study 2.

BP, Baseline period; IP, Intervention period; FU, Follow-up

Primary outcome

Action Research Arm Test (ARAT)

As no suitable measure of arm motor function has been validated for children with NMD, the functional abilities of the upper extremity (reaching, lifting and manipulating) are assessed bilaterally with the Action Research Arm Test (ARAT).[40] The ARAT is a standardized tool for the assessment of arm motor function and capacity (both distally and proximally) and consists of 19 items in four dimensions: grasp, grip, pinch and gross movements. The ARAT has shown to be reliable[40,41], valid[40,41,42] and suitable to detect changes over time[42] in stroke patients. The ARAT is performed and scored following standardized procedures as described by Yazbatiran.[41] The non-dominant arm is assessed both with and without arm support.

Secondary outcomes

Table 2 shows an overview of the secondary study outcomes and their psychometric properties. Both the dominant and non-dominant arms are assessed. The non-dominant arm is assessed both with and without arm support.

Adverse events

All adverse events will be recorded. The study will be terminated prematurely for an individual participant when training is excessive and remains excessive after a reduction of the training intensity. Signs for overstrain are: excessive muscle pain during the training, prolonged post-exercise muscle pain, a severely uncomfortable feeling during or after the training and extreme (muscle) fatigue (OMNI scale > 6).

Sample size

This is a first explorative study that will give an indication of the effect of functional training with arm support on the functional abilities of the upper extremity in boys with DMD. In this perspective, no realistic power calculation can be made. By using a design with multiple measurements at baseline we anticipate to find statistically significant effects of the proposed therapeutic intervention in ten boys with DMD.

Analysis

A one-way analysis of variance (ANOVA) of time will be used to examine the effects of functional arm training with arm support on the primary outcome (ARAT). Secondary outcomes will be treated similarly or by non-parametric analyses dependent on the type of outcome (interval or ordinal). Data will be analyzed with SPSS version 16.0 and an alpha level of 0.05 will be used to decide on statistical significance.

Discussion

This paper presents the research questions, hypotheses and methods of the No Use is Disuse (NUD) study in the Netherlands. The NUD study examines the effects of low-intensity dynamic physical training in boys at different stages in the course of Duchenne muscular dystrophy (DMD). It is expected that these dynamic exercises will be beneficial and will not impose a risk of muscle or other injury to the boys. This hypothesis is based on the (limited amount of) evidence that is available about physical training in children with neuromuscular diseases (NMD). In addition, the study was designed with the extensive input from several experts in the field of NMD in children (1 pediatric physiotherapist, 2 occupational therapists, 1 rehabilitation physician, 1 exercise physiologist, 1 neurologist, 1 clinical neurophysiologist and 1 epidemiologist). We will discuss the most relevant issues and decisions concerning 1) training (intensity), 2) outcome measures and 3) strategies to optimize therapy compliance.

Training (intensity)

Boys in both studies are instructed to train five days per week during 24 weeks. Although this training frequency is high, it was chosen to elicit as much 'daily physical activity' as possible in every boy. Many boys with DMD, especially those who are confined to a wheelchair, have a sedentary lifestyle[28] and do not engage in 60 min of moderate daily physical activity as recommended by the WHO for children to reduce their risk of chronic diseases.[43] On the other hand, as boys with DMD already have a chronic disease, their physical limitations have to be taken into account to prevent exercise-induced physical deterioration.[44] Nevertheless, the level of daily low-intensity physical activity as prescribed in this study is thought to be safe and effective in reducing disuse atrophy and excessive functional loss.

Although training-induced improvements of muscle functions can normally be expected after six weeks of physical training[26], we have chosen a training period of 24 weeks. This relatively long training period is considered necessary, as the disease progression is relatively slow, and the expected training effects over time probably small. We also assume that if boys are able to adhere to the training regime during these 24 weeks, it will be feasible for them to incorporate physical training in daily life afterwards.

Boys in study 2 train reaching and lifting movements, since reaching is one of the tasks with a high priority in potential users of an arm support.[45] Boys train their non-dominant arm as this arm is used less than the dominant arm in functional acitivities.[46] Therefore, training effects are expected to be larger for the non-dominant arm. Nevertheless, it may be difficult for boys to perform activities (like eating and scratching) with their non-dominant arm.

Study outcomes

In this study, reliable functional scales are used, such as the MFM, as well as timed tests that have clinically meaningful endpoints[47] and that are sufficiently sensitive to detect therapy related gains.[38] Indeed, outcome measures should be reliable and sensitive to allow for an adequate power in trials with a relatively small sample size. As no suitable ergometry test or arm motor function test is available for (non-ambulant) boys with NMD, a Six-Minute Bicycle test and the ARAT have been selected as primary outcomes. Pilot studies showed that the applied Six-Minute Bicycle test was feasible for both ambulant and recently wheelchair-dependent boys with DMD. The ARAT proved to be useful in a wheelchair-dependent boy with DMD as well (unpublished data). In addition, we used an upper extremity protocol to quantitatively measure arm motor function[48] with an eight camera motion analysis system (Vicon Motion Systems, Ltd, Oxford, UK). It has been shown that such a system can accurately measure task performance with an upper-limb orthosis.[45] Arm motor function has never been measured quantitatively before in boys with DMD, which may be of value because of its continuous properties and presumably greater sensitivity to change.[49]

Strategies to increase therapy compliance

From a previous study, it can be concluded that home-based cycling programs for children are feasible and show good adherence rates.[50] A home-based program allows boys to train at times that are convenient for them and reduces travel time. However, a home-based program requires great discipline from boys and their families, and several aspects need attention to optimize therapy compliance. One of these aspects, according to the Social Cognitive Theory[51], is that the social environment (e.g. family and school) is essential for a change in health behavior. Therefore, to optimize therapy compliance, parents are asked to stimulate their boys by reminding them to perform their exercises. In addition, siblings can be role models by being physically active themselves. Other strategies that are used to enhance behavioral change and optimize therapy compliance are specific goal setting[52] and reduction of perceived barriers.[53] For this reason, as described previously, all boys sign a training contract including an agreement on the training moments. The barriers that boys or their parents might perceive are discussed beforehand. In addition, the boys in study 1 are recommended to train while watching the television to make the training more pleasurable.

Expected products

The NUD study is the first clinical trial that examines the effects of low-intensity physical training on muscle endurance and functional abilities in boys with DMD. It will be a start to fill the current gap in our knowledge about the efficacy of physical training in these boys and will increase our insight into what type of physical training should be recommended. Although the NUD study focuses on children with DMD, the results may

be (partly) applicable to other neuromuscular disorders in childhood. Results of the NUD study are expected by the end of 2010.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

IJMG and NA handled funding and designed the first draft of the study protocol. ACHG was responsible for supervision of the project. All authors developed and wrote the final study protocol (drafted by MJ, critically revised by IJMG, NA and ACHG) and gave approval of the version to be published.

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References

1. van Essen AJ, Busch HF, te Meerman GJ, ten Kate LP. Birth and population prevalence of Duchenne muscular dystrophy in The Netherlands. *Hum Genet* 1992;88:258-266.
2. Hoffman EP, Brown RH, Kunkel LM. Dystrophin: the protein product of the Duchene muscular dystrophy locus. 1987. *Biotechnology* 1992;24:457-466.
3. Kanagawa M, Toda T. The genetic and molecular basis of muscular dystrophy: roles of cell-matrix linkage in the pathogenesis. *J Hum Genet* 2006;51:915-926.
4. McDonald CM, Abresch RT, Carter GT et al. Profiles of neuromuscular diseases. Duchenne muscular dystrophy. *Am J Phys Med Rehabil* 1995;74:S70-S92.
5. Eagle M, Bourke J, Bullock R et al. Managing Duchenne muscular dystrophy--the additive effect of spinal surgery and home nocturnal ventilation in improving survival. *Neuromuscul Disord* 2007;17:470-475.
6. Eagle M. Report on the muscular dystrophy campaign workshop: exercise in neuromuscular diseases Newcastle, January 2002. *Neuromuscul Disord* 2002;12:975-983.
7. Bar-or O, Rowland TW. Neuromuscular and musculoskeletal diseases. In: *Pediatric Exercise Medicine From Physiologic Principles to Health Care Application*. Human Kinetics; 2004; p. 269-303.
8. McDonald CM: Physical activity, health impairments, and disability in neuromuscular disease. *Am J Phys Med Rehabil* 2002;81:S108-S120.
9. Quinlivan R, Roper H, Davie M, Shaw NJ, McDonagh J, Bushby K. Report of a Muscular Dystrophy Campaign funded workshop Birmingham, UK, January 16th 2004. Osteoporosis in Duchenne muscular dystrophy; its prevalence, treatment and prevention. *Neuromuscul Disord* 2005;15:72-79.
10. McDonald DG, Kinali M, Gallagher AC. Fracture prevalence in Duchenne muscular dystrophy. *Dev Med Child Neurol* 2002;44:695-698.
11. Vestergaard P, Glerup H, Steffensen BF, Rejnmark L, Rahbek J, Moseklide L. Fracture risk in patients with muscular dystrophy and spinal muscular atrophy. *J Rehabil Med* 2001;33:150-155.
12. de Lateur BJ, Giaconi RM. Effect on maximal strength of submaximal exercise in Duchenne muscular dystrophy. *Am J Phys Med* 1979;58:26-36.
13. Scott OM, Hyde SA, Goddard C, Jones R, Dubowitz V. Effect of exercise in Duchenne muscular dystrophy. *Physiotherapy* 1981;67:174-176.
14. Vignos PJ, Jr., Watkins MP: The effect of exercise in muscular dystrophy. *JAMA* 1966; 197:843-848.
15. Dupont-Versteegden EE, McCarter RJ, Katz MS. Voluntary exercise decreases progression of muscular dystrophy in diaphragm of mdx mice. *J Appl Physiol* 1994.;77:1736-1741.
16. Hayes A, Williams DA. Beneficial effects of voluntary wheel running on the properties of dystrophic mouse muscle. *J Appl Physiol* 1996;80:670-679.
17. Wineinger MA, Abresch RT, Walsh SA, Carter GT. Effects of aging and voluntary exercise on the function of dystrophic muscle from mdx mice. *Am J Phys Med Rehabil* 1998;77:20-27.
18. Hayes A, Williams DA. Contractile function and low-intensity exercise effects of old dystrophic (mdx) mice. *Am J Physiol* 1998;274:C1138-C1144.
19. Carter GT, Abresch RT, Fowler WM, Jr. Adaptations to exercise training and contraction-induced muscle injury in animal models of muscular dystrophy. *Am J Phys Med Rehabil* 2002; 81:S151-S161.
20. Eagle M, Baudouin SV, Chandler C, Giddings DR, Bullock R, Bushby K. Survival in Duchenne muscular dystrophy: improvements in life expectancy since 1967 and the impact of home nocturnal ventilation. *Neuromuscul Disord* 2002;12:926-929.
21. Petrof BJ. The molecular basis of activity-induced muscle injury in Duchenne muscular dystrophy. *Mol Cell Biochem* 1998;179:111-123.
22. Lim JH, Kim DY, Bang MS. Effects of exercise and steroid on skeletal muscle apoptosis in the mdx mouse. *Muscle Nerve* 2004;30:456-462.
23. Okano T, Yoshida K, Nakamura A et al. Chronic exercise accelerates the degeneration-regeneration cycle and downregulates insulin-like growth factor-1 in muscle of mdx mice. *Muscle Nerve* 2005;32:191-199.
24. Kaczor JJ, Hall JE, Payne E, Tarnopolsky MA. Low intensity training decreases markers of oxidative stress in skeletal muscle of mdx mice. *Free Radic Biol Med* 2007;43:145-154.

25. Webster C, Silberstein L, Hays AP, Blau HM. Fast muscle fibers are preferentially affected in Duchenne muscular dystrophy. *Cell* 1988;52:503-513.
26. Voet NB, van der Kooi EL, Riphagen II, Lindeman E, van Engelen BG, Geurts AC. Strength training and aerobic exercise training for muscle disease. *Cochrane Database Syst Rev* 2010;CD003907.
27. Robertson RJ, Goss FL, Boer NF et al. Children's OMNI scale of perceived exertion: mixed gender and race validation. *Med Sci Sports Exerc* 2000;32:452-458.
28. McDonald CM, Widman LM, Walsh DD. Use of step activity monitoring for continuous physical activity assessment in boys with Duchenne muscular dystrophy. *Arch Phys Med Rehabil* 2005;86:802-808.
29. World Health Organization. *ICF: Internaional Classification of Functiononing, Disability and Health*. Geneva 2001.
30. Tirosh E, Bar-Or O, Rosenbaum P. New muscle power test in neuromuscular disease. Feasibility and reliability. *Am J Dis Child* 1990;144:1083-1087.
31. Kallenberg LA, Schulte E, sselhorst-Klug C, Hermens HJ. Myoelectric manifestations of fatigue at low contraction levels in subjects with and without chronic pain. *J Electromyogr Kinesiol* 2007;17:264-274.
32. Zwarts MJ, Bleijenberg G, van Engelen BG. Clinical neurophysiology of fatigue. *Clin Neurophysiol* 2008;119:2-10.
33. Gosselin N, Lambert K, Poulain M, Martin A, Prefaut C, Varray A. Endurance training improves skeletal muscle electrical activity in active COPD patients. *Muscle Nerve* 2003;28:744-753.
34. Oliveira AS, Goncalves M. EMG amplitude and frequency parameters of muscular activity: effect of resistance training based on electromyographic fatigue threshold. *J Electromyogr Kinesiol* 2009;19:295-303.
35. Hermens HJ, Freriks B, sselhorst-Klug C, Rau G. Development of recommendations for SEMG sensors and sensor placement procedures. *J Electromyogr Kinesiol* 2000;10:361-374.
36. Pfeiffer KA, Pivarnik JM, Womack CJ, Reeves MJ, Malina RM. Reliability and validity of the Borg and OMNI rating of perceived exertion scales in adolescent girls. *Med Sci Sports Exerc* 2002,34:2057-2061.
37. Berard C, Payan C, Fermanian J, Girardot F. A motor function measurement scale for neuromuscular diseases - description and validation study. *Rev Neurol* 2006;162:485-493.
38. Vuillerot C, Girardot F, Payan C et al. Monitoring changes and predicting loss of ambulation in Duchenne muscular dystrophy with the Motor Function Measure. *Dev Med Child Neurol* 2010;52:60-65.
39. Jannink MJ, van der Wilden GJ, Navis DW, Visser G, Gussinklo J, Ijzerman M. A low-cost video game applied for training of upper extremity function in children with cerebral palsy: a pilot study. *Cyberpsychol Behav* 2008;11:27-32.
40. Lyle RC. A performance test for assessment of upper limb function in physical rehabilitation treatment and research. *Int J Rehabil Res* 1981;4:483-492.
41. Yozbatiran N, Der-Yeghiaian L, Cramer SC. A standardized approach to performing the Action Research Arm Test. *Neurorehabil Neural Repair* 2008;22:78-90.
42. Hsieh YW, Wu CY, Lin KC, Chang YF, Chen CL, Liu JS. Responsiveness and validity of three outcome measures of motor function after stroke rehabilitation. *Stroke* 2009;40:1386-1391.
43. Strong WB, Malina RM, Blimkie CJ et al. Evidence based physical activity for school-age youth. *J Pediatr* 2005;146:732-737.
44. Morris PJ. Physical activity recommendations for children and adolescents with chronic disease. *Curr Sports Med Rep* 2008;7:353-358.
45. Romilly DP, Angelin C, Gosini RG, Hershler C, Raschka SU. A Functional Task Analysis and Motion Simulation for the Development of a Powered Upper-Limb Orthosis. *IEEE Transactions on Rehabilitation Engineering* 1994;2:119-129.
46. Hiller LB, Wade CK. Upper extremity functional assessment scales in children with Duchenne muscular dystrophy: a comparison. *Arch Phys Med Rehabil* 1992;73:527-534.
47. Main M, Kairon H, Mercuri E, Muntoni F. The Hammersmith functional motor scale for children with spinal muscular atrophy: a scale to test ability and monitor progress in children with limited ambulation. *Eur J Paediatr Neurol* 2003;7:155-159.
48. Cutti AG, Paolini G, Troncossi M, Cappello A, Davalli A. Soft tissue artefact assessment in humeral axial rotation. *Gait Posture* 2005;21:341-349.
49. Scott E, Mawson SJ. Measurement in Duchenne muscular dystrophy: considerations in the development of a neuromuscular assessment tool. *Dev Med Child Neurol* 2006;48:540-544.

50. Johnston TE, Smith BT, Oladeji O, Betz RR, Lauer RT. Outcomes of a home cycling program using functional electrical stimulation or passive motion for children with spinal cord injury: a case series. *J Spinal Cord Med* 2008;31:215-221.
51. Bandura A. Social cognitive theory: an agentic perspective. *Annu Rev Psychol* 2001;52:1-26.
52. Strecher VJ, Seijts GH, Kok GJ et al. Goal setting as a strategy for health behavior change. *Health Educ Q* 1995;22:190-200.
53. Janz NK, Becker MH. The Health Belief Model: a decade later. *Health Educ Q* 1984;11:1-47.
54. Norkin CC, White DJ: *Measurement of Joint Motion: A Guide to Goniometry.* F.A. Davis Compagny; 2003.
55. Brooke MH, Griggs RC, Mendell JR, Fenichel GM, Shumate JB, Pellegrino RJ. Clinical trial in Duchenne dystrophy. I. The design of the protocol. *Muscle Nerve* 1981;4:186-197.
56. Florence JM, Pandya S, King WM et al. Clinical trials in Duchenne dystrophy. Standardization and reliability of evaluation procedures. *Phys Ther* 1984;64:41-45.
57. Wagner MB, Vignos PJ, Jr., Carlozzi C, Hull AL. Assessment of hand function in Duchenne muscular dystrophy. *Arch Phys Med Rehabil* 1993;74:801-804.
58. Florence JM, Pandya S, King WM et al. Intrarater reliability of manual muscle test (Medical Research Council scale) grades in Duchenne's muscular dystrophy. *Phys Ther* 1992;72:115-122.
59. Escolar DM, Henricson EK, Mayhew J et al. Clinical evaluator reliability for quantitative and manual muscle testing measures of strength in children. *Muscle Nerve* 2001;24:787-793.
60. Scholten RR, Pillen S, Verrips A, Zwarts MJ. Quantitative ultrasonography of skeletal muscles in children: normal values. *Muscle Nerve* 2003;27:693-698.
61. Pillen S, Keimpema van M, Nievelstein RA et al. Skeletal muscle ultrasonography: Visual versus quantitative evaluation. *Ultrasound Med Biol* 2006,32:1315-1321.
62. Pillen S, Verrips A, Alfen van N, Arts IM, Sie LT, Zwarts MJ. Quantitative skeletal muscle ultrasound: diagnostic value in childhood neuromuscular disease. *Neuromuscul Disord* 2007;17:509-516.
63. Leonard CM, Roza MA, Barr RD, Webber CE. Reproducibility of DXA measurements of bone mineral density and body composition in children. *Pediatr Radiol* 2009;39:148-154.
64. Lohman M, Tallroth K, Kettunen JA, Marttinen MT. Reproducibility of dual-energy x-ray absorptiometry total and regional body composition measurements using different scanning positions and definitions of regions. *Metabolism* 2009;58:1663-1668.
65. Mendell JR, Moxley RT, Griggs RC et al. Randomized, double-blind six-month trial of prednisone in Duchenne's muscular dystrophy. *N Engl J Med* 1989;320:1592-1597.
66. Mattar FL, Sobreira C. Hand weakness in Duchenne muscular dystrophy and its relation to physical disability. *Neuromuscul Disord* 2008;18:193-198.
67. Graham HK, Harvey A, Rodda J, Nattrass GR, Pirpiris M. The Functional Mobility Scale (FMS). *J Pediatr Orthop* 2004;24:514-520.
68. Harvey A, Graham HK, Morris ME, Baker R, Wolfe R. The Functional Mobility Scale: ability to detect change following single event multilevel surgery. *Dev Med Child Neurol* 2007;49:603-607.
69. Angelini C. The role of corticosteroids in muscular dystrophy: a critical appraisal. *Muscle Nerve* 2007;36:424-435.
70. Mayhew JE, Florence JM, Mayhew TP et al. Reliable surrogate outcome measures in multicenter clinical trials of Duchenne muscular dystrophy. *Muscle Nerve* 2007;35:36-42.
71. Beenakker EA, Maurits NM, Fock JM, Brouwer OF, van der Hoeven JH. Functional ability and muscle force in healthy children and ambulant Duchenne muscular dystrophy patients. *Eur J Paediatr Neurol* 2005;9:387-393.
72. Mathiowetz V., Weber G VG, Kashman N. Adult norms for the nine-hole peg test of finger dexterity. *Occup Ther J Res* 1985;5:24-38.
73. Smith YA, Hong E, Presson C: Normative and validation studies of the Nine-hole Peg Test with children. *Percept Mot Skills* 2000, 90:823-843.
74. Jebsen RH, Taylor N, Trieschmann RB, Trotter MJ, Howard LA. An objective and standardized test of hand function. *Arch Phys Med Rehabil* 1969;50:311-319.
75. Coster J, Haley SM. Conceptualization and measurement of disablement in infants and young children. Inf Young Children 1992;4:11-22.
76. Custers JW, Wassenberg-Severijnen JE, van der Net J, Vermeer A, Hart HT, Helders PJ. Dutch adaptation and content validity of the 'Pediatric Evaluation Of Disability Inventory (PEDI)'. *Disabil Rehabil* 2002;24:250-258.

77. Wassenberg-Severijnen JE, Custers JW, Hox JJ, Vermeer A, Helders PJ. Reliability of the Dutch Pediatric Evaluation of Disability Inventory (PEDI). *Clin Rehabil* 2003;17:457-462.
78. Custers JW, van der Net J, Hoijtink H, et al. Discriminative validity of the Dutch Pediatric Evaluation of Disability Inventory. *Arch Phys Med Rehabil* 2002;83:1437-1441.
79. Vos-Vromans DC, Ketelaar M, Gorter JW. Responsiveness of evaluative measures for children with cerebral palsy: the Gross Motor Function Measure and the Pediatric Evaluation of Disability Inventory. *Disabil Rehabil* 2005;27:1245-1252.
80. Penta M, Thonnard JL, Tesio L. ABILHAND: a Rasch-built measure of manual ability. *Arch Phys Med Rehabil* 1998;79:1038-1042.
81. Arnould C, Penta M, Renders A, Thonnard JI. ABILHAND-Kids: a measure of manual ability in children with cerebral palsy. *Neurology* 2004;63:1045-52.
82. Durez P, Fraselle V, Houssiau F, Thonnard JL, Nielens H, Penta M. Validation of the ABILHAND questionnaire as a measure of manual ability in patients with rheumatoid arthritis. *Ann Rheum Dis* 2007;66:1098-1105.
83. Johnson LM, Randall MJ, Reddihough DS, Oke LE, Byrt TA, Bach TM. Development of a clinical assessment of quality of movement for unilateral upper-limb function. *Dev Med Child Neurol* 1994;36:965-973.
84. Randall M, Carlin JB, Chondros P, Reddihough D. Reliability of the Melbourne assessment of unilateral upper limb function. *Dev Med Child Neurol* 2001;43:761-767.
85. Bourke-Taylor H. Melbourne Assessment of Unilateral Upper Limb Function: construct validity and correlation with the Pediatric Evaluation of Disability Inventory. *Dev Med Child Neurol* 2003;45:92-96.
86. Ravens-Sieberer U, Gosch A, Rajmil L et al. KIDSCREEN-52 quality-of-life measure for children and adolescents. *Expert Rev Pharmacoecon Outcomes Res* 2005;5:353-364.
87. Ravens-Sieberer U, Gosch A, Rajmil L et al. The KIDSCREEN-52 Quality of Life Measure for Children and Adolescents: Psychometric Results from a Cross-Cultural Survey in 13 European Countries. *Value Health* 2007;11:645-658.
88. Young B, Rice H, xon-Woods M, Colver AF, Parkinson KN. A qualitative study of the health-related quality of life of disabled children. *Dev Med Child Neurol* 2007;49:660-665.
89. Crocker PR, Bailey DA, Faulkner RA, Kowalski KC, McGrath R. Measuring general levels of physical activity: preliminary evidence for the Physical Activity Questionnaire for Older Children. *Med Sci Sports Exerc* 1997;29:1344-1349.
90. Prochaska JJ, Sallis JF, Long B. A physical activity screening measure for use with adolescents in primary care. *Arch Pediatr Adolesc Med* 2001;155:554-559.

6

Assisted bicycle training delays functional deterioration in boys with Duchenne muscular dystrophy: the randomized controlled trial "No Use is Disuse"

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Abstract

Background: Physical training might delay the functional deterioration caused by disuse in boys with Duchenne muscular dystrophy (DMD). The No Use is Disuse (NUD) study is the first explorative, randomized controlled trial (RCT) in boys with DMD to examine whether assisted bicycle training is feasible, safe and beneficial.

Methods: Ambulatory and recently wheelchair-dependent boys with DMD were allocated to the intervention or control group. The intervention group received assisted bicycle training of the legs and arms during 24 weeks. The control group received the same training after a waiting period of 24 weeks. The primary study outcomes were the Motor Function Measure (MFM) and the Assisted Six-Minute Cycling Test (A6MCT). Group differences were examined by an analysis of covariance (ANCOVA).

Results: Thirty boys (mean age 10.5±2.6 year, 18 ambulant and 12 wheelchair-dependent) were allocated to the intervention (n=17) or the control (n=13) group. All boys in the intervention group (except one) completed the training. After 24 weeks the total MFM score remained stable in the intervention group, whereas it had significantly decreased in the control group (Δ=4.9, 95%CI=2.2 to 7.6). No significant group differences were found for the A6MCT. No serious adverse events were observed.

Conclusions: Our results suggest that assisted bicycle training of the legs and arms is feasible and safe for both ambulant and wheelchair-dependent children and may decline the deterioration due to disuse. Progressive deterioration, however, may compromise the design of trials for DMD.

Introduction

Duchenne muscular dystrophy (DMD) is the most common debilitating neuromuscular disorder affecting 1/3500 live-born boys.[1] Although symptomatic treatments, such as corticosteroids and respiratory ventilation, have extended the life expectancy of boys with DMD into adulthood, boys still become wheelchair-dependent around the age of ten years and also lose their upper-limb functions.[2,3] Therefore an important aim in the management of DMD is to delay the loss of functional abilities, which is relevant for both activities of daily living (ADL) and physical aspects of health-related quality of life.

The loss of functional abilities is primarily the result of a progressive decrease in muscle strength and muscle endurance during the course of DMD.[4,5] However, increasingly limited physical and social activities gradually cause a discrepancy between a boy's potential capacities and his actual performance. For example, the increasing amount of energy needed to ambulate and the increasing risk of falling will make boys move less and make them opt for wheelchair mobility. Confinement to a wheelchair will lead to secondary deterioration of the musculoskeletal and cardiorespiratory systems.[5] Another example is the loss of arm function that is accelerated after the onset of wheelchair-dependency by forcing the boys to function within the confines of the wheelchair.[6] This leads to secondary physical deterioration or 'disuse'.

Physical training might delay the secondary functional deterioration as a result of disuse as long as eccentric exercises and exhausting high-resistance exercises are avoided.[7,8] Current international guidelines therefore recommend regular sub-maximal activities for boys with DMD. [9] However, the number of randomized controlled trials (RCTs) that have examined the effects of physical training in muscle diseases is limited to three trials conducted in adults.[10] Based on these trials, no specific training advice can be given. Even fewer studies have examined the effects of physical training in boys with DMD and none of these were conducted using a controlled design. These studies mainly focused on resistance exercises in ambulatory boys and concluded that although sub-maximal resistance exercises did not cause any harm, they had only limited beneficial effects.[11-13] In contrast, previous studies in mdx mice (an animal model for DMD) concluded that dynamic exercises (such as voluntary wheel-running) had beneficial effects on muscle strength and fatigue resistance.[14,15] Dynamic exercises (bicycle training) also improved endurance and strength in patients with Becker Muscular Dystrophy, a milder variant of DMD.[16] However, these patients were all adults. Therefore no RCTs have been published concerning the effects of dynamic, active-assisted training in children with DMD.

Based on previous studies in adults and mdx mice and on clinical experience, it was hypothesized that a dynamic physical training would be feasible and safe and would

counteract the secondary functional decline caused by disuse in boys with DMD. An RCT was therefore conducted that compared assisted bicycle training with no intervention (usual care) in children who were at the end of their ambulatory phase or who had recently been confined to a wheelchair: the No Use is Disuse (NUD) study.[17] We chose assisted bicycle training instead of bicycle training without support, because even the smallest resistance can be too hard to sustain for patients in whom large muscle groups are affected.[18] This is also the first study to use assisted training in DMD, since previous (uncontrolled) trials all examined the effect of resistance training.

Methods

An extensive description of the NUD study has previously been published.[17] No deviations from the original protocol were made.

Participants

An RCT with multiple baseline measurements was conducted between January 2009 and January 2011 among boys with a DNA-established diagnosis of DMD. Boys were eligible if they were in their late ambulatory phase and showed a labored gait and/or had difficulties with rising from the floor. Boys were included if they needed more than 5 seconds to rise from the floor, or were not able to rise from the floor, or were not able to cycle without electric assistance, or needed a wheelchair to move over a long (>500m) distance. Wheelchair-dependent boys were eligible if they were able to touch the top of their head with both hands or were able to use a hand-operated wheelchair. Exclusion criteria were: age <6 years, a clinical cardiomyopathy, and other disabling diseases influencing mobility. Approval was obtained from the regional Medical Ethics Committee. Parents and participants who were over 12 years of age provided informed consent. The trial was registered with the Netherlands National Trial Register, number 1631.

Randomization and masking

Stratified randomization (ambulant versus wheelchair-dependent) was used to allocate participants to either the intervention or the control group in a 2:1 ratio. After a baseline period of 8 weeks, the intervention group received assisted bicycle training, whereas the control group received usual care during 24 weeks. Usual care means that boys were allowed to continue their activities including physiotherapy, but they did not receive any specific intervention. The control group received the same training after the 24-week waiting period. The intervention and control group were followed up until 56 and 60 weeks after study entry, respectively. Participants and outcome assessor were not blinded to treatment allocation, but had no information about previous test results at each assessment.

Sample size

No historical data were available at the start of this study in 2008.[19] The sample size was therefore not based on statistical analysis. We arbitrarily chose to include 20 to 30 participants. This seemed a realistic number of participants to recruit in the Netherlands in view of an estimated number of ~100 eligible boys with DMD aged 7 to 13 years old. Boys were recruited from the Dutch Duchenne Parent Project (DPP) database and by advertisements on the websites of the Dutch Association for Neuromuscular Diseases (VSN) and DPP between January 2009 and January 2010.

Procedures

Intervention

The intervention consisted of an assisted-bicycle-training program of five days per week during 24 weeks. In each session, participants cycled 15 minutes with both their legs and arms using a mobility trainer with electrical motor support (KPT Cycla, Kinetic, France). The mobility trainer could be used with a participant's own (electric) wheelchair. We chose assisted bicycle training instead of bicycle training without support, because even the smallest resistance can be too hard to sustain for patients in whom large muscle groups are affected (assistance was at least 6 rounds per minute if no active movement).[18] Participants were instructed to cycle at a constant speed (~65 RPM) and to keep this up for 15 minutes without getting overexerted as assessed with the OMNI scale for perceived exertion (OMNI>6).[20] This means that participants had to be able to cycle at an exertion ranging from 'a little tired' to 'getting more tired'. The mobility trainer recorded the time and number of revolutions. Starting positions for both leg-cycling and arm-cranking were standardized[21], i.e. for leg-cycling the hip and knee of the bended leg were held in 90° flexion, while the knee of the other leg was submaximally extended. The feet were placed in a heel cup with straps. For arm cycling, the pedal axis was a few centimeters (with a maximum of 5 centimeters) below shoulder level when the pedals were horizontal. The distance from the chair to the bicycle was decided by allowing the participants to move their legs and arms over the submaximal range of motion, which was allowed to produce a feeling of stretch but not pain. Participants were seated in a comfortable position with the back supported and they were recommended to watch television while cycling to make training more pleasurable. Participants trained at home or at school (depending on their preferences). Parents and/or teachers were instructed to assist the boys. Training intensity and posture were monitored and if necessary adjusted by the primary investigator (MJ).

Assessments

Figure 1 shows the subsequent clinical assessments (T0–T8) with the primary endpoint at T4. Multiple baseline measurements (T0-T2) were performed to control for initial severity and disease progression. All assessments were performed by the primary investigator (MJ)

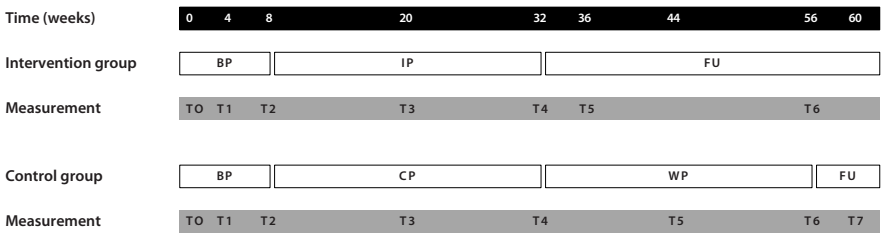

Figure 1 Clinical assessments during the study period.

BP, Baseline period; IP, Intervention period; FU, Follow-up; CP, Control period

at a university hospital (T0, T2, T4, T6), but also at home (other assessments) to reduce the burden for the participants and families.

Outcome measures

The primary outcome measures were the MFM[22] and the Assisted Six-Minute Cycling Test (A6MCT).[21] The MFM assesses the functional abilities and consists of 32 items in 3 dimensions: Dimension 1= standing position and transfers (D1); Dimension 2= axial and proximal motor function (D2); Dimension 3= distal motor function (D3). We calculated the percentage of the maximum score for the total score and for the three dimensions separately, with a higher score indicating a better motor function. The A6MCT for both the legs and the arms was used to assess endurance (at T0, T2, T4 and T6), in which participants were instructed to make as many revolutions as possible in 6 minutes.

Secondary outcome measures were the Pediatric Evaluation of Disability Inventory (PEDI)[23], timed tests (rise from floor, 10m run, nine-hole peg test), muscle strength, passive joint range of motion (ROM) and quantitative muscle ultrasound (QMUS). Muscle strength of the hip extensors, knee extensors, ankle dorsiflexors, shoulder abductors and elbow extensors was scored bilaterally with the Medical Research Council (MRC) scale[24]. We calculated a total sum score, a sum score for the lower limb, and a sum score for the upper limb. ROM was recorded bilaterally for knee extension, ankle dorsiflexion and elbow extension and we calculated a sum score for each joint.[25] QMUS was used to calculate the echo intensity (EI) of the biceps brachii muscle, the forearm flexors, the rectus femoris muscle and the tibialis anterior muscle.[26] The EI is a grey value quantified by a histogram-based analysis that shows the infiltration of connective tissue and fat. In boys with DMD, muscle EI increases with age and disease severity.[27] EI's were expressed as z-scores (i.e. the number of SD from the mean of the reference group) and were then integrated in a total sum score, and a sum score for the lower and upper limb. Age (years), co-interventions (e.g. physiotherapy), and corticosteroid use (in the Netherlands: 10 days on 10 days off schedule of 0.75 mg/kg) were recorded as well.

Adverse events

During the intervention period, participants who received the assisted bicycle training were monitored for signs of overexertion by means of a postal questionnaire once every two weeks and home visits by the primary investigator (MJ). The questionnaire focused on excessive muscle pain, any severely uncomfortable feeling during or after the training, and (extreme) fatigue. Parents and their boys were informed by telephone when compliance was insufficient or when other problems (such as signs of overstrain) were identified. Parents were also instructed to call or email the investigator if their boys had significant pain or fatigue. In that case, the training intensity was adjusted. The study terminated prematurely for a participant if training remained excessive after a reduction of the training intensity.

Statistical analysis

Participants were analyzed as treated. Group differences for the primary outcome measures were examined by an analysis of covariance (ANCOVA), using group (intervention versus control) as the independent factor, and the multiple baseline assessments (T0-T2), corticosteroid use, and ambulatory status as covariates. Multiple baseline assessments (T0-T2) were added as covariates, as paired *t*-tests showed small changes in total MFM score over time already between T0 and T1 (-2.7 (4.3), 95%CI=0.09 to 5.30), and T0 and T2 (-2.9 (3.6), 95%CI=0.74 to 5.11) for the control group, and also between T1 and T2 (1.7 (2.3), 95%CI=0.39 to 3.04), and T0 and T2 (-2.5 (2.0), 95%CI=1.41 to 3.66) for the intervention group. However, a high degree of correlation was found between T0 and T2 for both the control (Intraclass Correlation Coefficient (ICC) = 0.96, 95%CI=0.88 to 0.99) and intervention (ICC=0.99, 95%CI=0.97 to 0.99) groups.

The same ANCOVA statistics were performed to establish effects for the individual dimensions of the MFM, the primary outcomes at T3 and the secondary outcomes at T3 and T4. Effect sizes (ES) were calculated to investigate the clinical relevance of the observed differences by dividing the group differences between the time effect T2-T4 by the pooled SD at T2. Interpretation of the ES is: 0.20-0.50: small; 0.50-0.80: moderate; and ≥0.80: large.[28] Non-parametric Wilcoxon rank-sum tests were used to evaluate the effect of training on the time to rise from floor and 10m run at T4, as these data did not show a normal distribution and groups were small because only a limited number of boys were able to perform the tests. Boys who were unable to perform the tests were coded with an unrealistically high score (100 seconds). In addition, 2 boys lost the ability to walk 10m and to rise from the floor in the control group, and 1 boy lost the ability to walk 10m and to rise from the floor in the intervention group during the study period.

Changes during the follow-up period for the intervention group were determined by comparing means at the end of the training to those 4 weeks (short-term) and 24 weeks later (long-term) using paired *t*-tests. Paired *t*-tests were also used to calculate the changes during the training period of the control group. All statistical analyses were performed

using SPSS v. 16.0 for Windows (SPSS Inc., Chicago, IL, USA) with a significance level set at $p<0.05$.

Results

Participants

Figure 2 shows the participant flow through the study. Thirty boys (18 ambulant, 12 wheelchair-dependent) were allocated to the intervention (n=17) or control group (n=13). Two out of the thirty wheelchair-dependent boys were not randomized, but directly allocated to the intervention group, because the study duration was ending. Another two

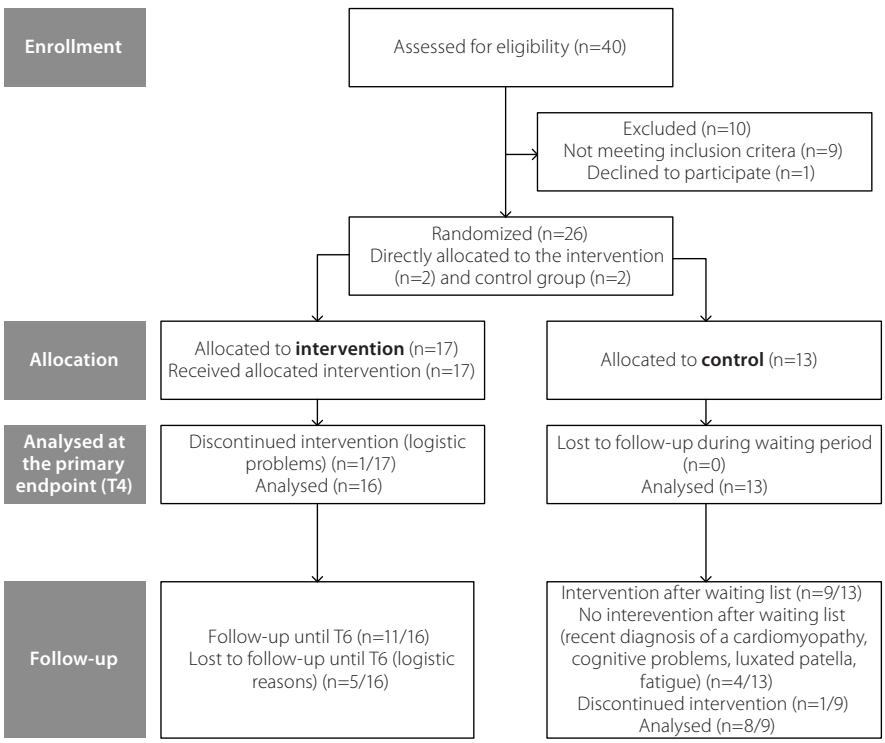

Figure 2 Participant flow through the study.

This figure shows the participant flow through the study. Thirty boys were allocated to the intervention (n=17) or control group (n=13). Of the 17 boys in the intervention group, only one participant discontinued the training and he was excluded from the analysis. No patients from the control group were lost to follow-up during the waiting period.

(ambulant) boys were originally allocated to the intervention group, but were replaced to the control group within two weeks after trying the intervention. The intervention appeared to be unfeasible for them due to cognitive problems for one young boy, and motivation problems and a patella luxation for another boy. Of the 17 boys in the intervention group, only one wheelchair-dependent participant discontinued the training and the assessments after 12 weeks, due to logistic problems imposed on his family, and he was excluded from the analysis. No patients from the control group were lost to follow-up during the waiting period. The mean age in the intervention group was 10.8 (SD=2.4) years and in the control group 10.5 (SD=2.8) years (p=0.803) at baseline. Eight boys were ambulant and 9 were wheelchair-dependent in the intervention group, compared to 10 and 3 in the control group respectively (p=0.098). In the intervention group 14 boys used corticosteroids compared to 9 in the control group. The clinical characteristics did not differ substantially between the two groups at baseline (T0-T2) (Table 1).

Feasibility and compliance

The 16 participants of the intervention group trained on average four (range 3-5) times per week) and achieved 550-1050 (mean~800) cycling revolutions with the legs (median OMNI score 3) and 600-1100 (mean~800) revolutions with the arms (median OMNI score 3). One wheelchair-dependent participant (total MFM score 47%) was able to perform the bicycle training with the arms only. Three boys only participated in arm bicycle training due to a lack of time or a limited attention span. One boy cycled only 6 instead of 15 minutes with his arms due to exhaustion (OMNI>6), even with maximal assistance.

Primary outcome measures T2-T4

The functional abilities were 4.9% higher in the intervention than in the control group at T4, after correcting for the ambulatory status and corticosteroid use at T2 and for the total MFM score at T0-T2 (Δ 4.9, 95%CI=2.2 to 7.6). This means that the total MFM score remained stable in the intervention group, whereas it decreased in the control group. In the intervention group, the same results were found for the D1 (*Δ*=7.0, 95%CI=2.0 to 11.9) and D3 (*Δ*=5.4, 95%CI=0.9 to 9.9), but not for the D2 of the MFM. The D3 already showed a training effect at T3 (*Δ*=5.8, 95%CI=0.8 to 10.8). Overall, the intervention group showed an ES 0.5 for the total MFM scores, an ES 0.4 for the D1, and an ES 0.8 for the D3. No training effects were found for the A6MCT for the legs (*Δ*=5.6, 95%CI=-56.2 to 67.3) and the arms (*Δ*=13.8, 95%CI= -60.7 to 88.3). (Table 1, Figure 3)

Table 1 Between-group differences at T3 and T4 (primary endpoint)

		Mean (SD) Baseline period		
		T0	**T1**	**T2**
Primary outcome measures				
MFM (%)				
- Total	Intervention	65.1 (15.6) n=15	66.8 (13.3) n=14	64.8 (15.0) n=17
	Control	70.9 (13.7) n=13	68.1 (15.3) n=13	67.9 (13.4) n=13
- D1	Intervention	26.0 (29.7) n=15	28.2 (28.4) n=14	28.1 (27.8) n=17
	Control	43.0 (27.3) n=13	37.9 (25.9) n=13	36.9 (24.3) n=13
- D2	Intervention	92.2 (13.8) n=16	94.4 (7.7) n=16	90.0 (12.7) n=17
	Control	90.0 (9.0) n=13	87.0 (13.9) n=13	87.6 (10.9) n=13
- D3	Intervention	92.3 (7.4) n=16	92.3 (5.1) n=16	89.9 (7.9) n=17
	Control	90.1 (7.6) n=13	91.9 (7.6) n=13	91.6 (6.8) n=13
A6MCT (Revolutions)				
- Legs	Intervention	434.7 (164.8) n=12	NA	397.1 (143.8) n=16
	Control	405.4 (113.2) n=8	NA	415.2 (158.3) n=13
- Arms	Intervention	403.0 (111.7) n=16	NA	378.7 (149.2) n=16
	Control	351.5 (110.7) n=8	NA	319 (122.9) n=12
Secondary outcome Measures				
PEDI (Scale scores)				
- Selfcare	Intervention	74.8 (13.2) n=16	NA	76.1 (13.4) n=17
	Control	72.3 (13.6) n=13	NA	72.9 (12.8) n=13
- Mobility	Intervention	57.6 (15.0) n=15	NA	59.0 (18.0) n=17
	Control	62.7 (11.0) n=13	NA	60.2 (13.2) n=13

	Mean (SD) Training/ Control period		Secondary endpoint: Between-group differences at T3*		Primary endpoint: Between-group differences at T4*	
	T3	**T4**	**Δ (95% CI)**	***p***	**Δ (95% CI)**	***p***
	64.3 (15.3) n=15	65.6 (16.9) n=16	-0.04 (-3.4 to 3.3)	0.979	4.9 (2.2 to 7.6)	0.002†
	65.4 (17.1) n=10	61.5 (13.0) n=13				
	27.4 (26.3) n=15	29.0 (29.2) n=16	0.9 (-5.2 to 7.0)	0.764	7.0 (2.0 to 11.9)	0.008†
	34.6 (28.2) n=10	27.8 (22.1) n=13				
	89.1 (15.7) n=16	90.3 (15.3) n=16	-4.1 (-8.6 to 0.5)	0.076	2.3 (-1.4 to 6.1)	0.211
	5.3 (15.7) n=10	83.5 (13.8) n=13				
	92.3 (8.7) n=16	91.4 (7.6) n=16	5.8 (0.8 to 10.8)	0.025†	5.4 (0.9 to 9.9)	0.021†
	88.6 (8.2) n=10	86.4 (8.0) n=13				
	NA	442.0 (124.3) n=14	NA	NA	5.6 (-56.2 to 67.3)	0.848
	NA	407.0 (187.4) n=11				
	NA	444.4 (107.6) n=14	NA	NA	13.8 (-60.7 to 88.3)	0.697
	NA	349.9 (131.9) n=9				
	NA	77.1 (14.1) n=15	NA	NA	4.1 (-2.2 to 10.4)	0.192
	NA	72.4 (14.7) n=11				
	NA	55.8 (13.0) n=15	NA	NA	1.6 (-3.6 to 6.0)	0.523
	NA	57.7 (15.2) n=11				

Table 1 Continued

		Mean (SD) Baseline period		
		T0	**T1**	**T2**
Secondary outcome Measures				
Timed tests (seconds)				
- Rise from floor	Intervention	6.3 (4.6) n=7	9.3 (9.6) n=7	9.3 (12.4) n=7
	Control	8.8 (5.3) n=8	11.5 (6.5) n=8	11.5 (5.8) n=8
- 10m run	Intervention	6.4 (1.7) n=6	NA	6.4 (2.3) n=7
	Control	8.2 (2.2) n=8	NA	8.8 (2.6) n=9
- 9HPT	Intervention	21.0 (4.3) n=16	19.6 (2.9) n=14	21.3 (4.9) n=15
	Control	24.6 (4.0) n=13	23.9 (5.6) n=13	22.9 (6.4) n=13
MRC (sum scores)				
- Total	Intervention	31.0 (5.5) n=14	31.4 (4.9) n=13	0.6 (6.0) n=13
	Control	31.4 (4.4) n=11	30.7 (5.1) n=13	29.3 (5.3) n=12
- Lower limb	Intervention	17.8 (4.5) n=15	17.3 (3.4) n=13	16.8 (4.0) n=13
	Control	17.5 (3.2) n=11	17.8 (3.0) n=13	17.2 (3.2) n=13
- Upper limb	Intervention	13.5 (2.4) n=15	14.1 (2.1) n=13	13.8 (2.7) n=13
	Control	13.3 (2.2) n=12	12.8 (2.5) n=13	12.4 (2.5) n=12
ROM (limitation in degrees)				
- Knee ext	Intervention	26.9 (27.4) n=16	21.2 (26.5) n=15	35.0 (37.5) n=12
	Control	6.8 (14.9) n=11	10.8 (26.2) n=13	7.1 (17.6) n=12
- Ankle dfl	Intervention	16.3 (25.2) n=16	20.0 (25.1) n=15	21.7 (30.6) n=12
	Control	5.9 (8.0) n=11	6.9 (12.5) n=13	7.9 (13.0) n=12
- Elbow ext	Intervention	15.3 (21.2) n=16	11.7 (14.8) n=15	15.0 (19.2) n=12
	Control	5.5 (10.4) n=11	8.1 (16.5) n=13	5.4 (9.9) n=12

Mean (SD) Training/ Control period		Secondary endpoint: Between-group differences at T3*		Primary endpoint: Between-group differences at T4*	
T3	**T4**	***Δ* (95% CI)**	***p***	***Δ* (95% CI)**	***p***
5.4 (2.4) n=6	4.8 (1.6) n=6	4·1	0·788‡	16.3	0.961‡
9.5 (7.0) n=5	21.1 (18.0) n=6				
NA	7.4 (3.9) n=8	NA	NA	-0.7‡	0.522‡
NA	7.3 (1.8) n=6				
20.0 (3.4) n=16	20.4 (5.0) n=16	-2.4 (-5.9 to 1.0)	0.153	0.6 (-3.1 to 2.4)	0.784
23.3 (5.7) n=10	22.4 (5.0) n=13				
30.5 (5.3) n=15	31.6 (1.4) n=14	1.3 (-0.5 to 3.0)	0.132	1.4 (-0.3 to 3.1)	0.098
31.4 (4.2) n=8	28.6 (5.7) n=12				
16.7 (5.1) n=15	17.6 (3.6) n=14	-0.2 (-0.8 to 1.2)	0.643	1.0 (-0.1 to 2.1)	0.082
18.0 (2.6) n=8	16.7 (3.6) n=12				
13.9 (2.2) n=15	13.7 (2.4) n=15	0.9 (0.0 to 1.8)	0.047†	0.8 (-0.1 to 1.7)	0.077
13.1 (1.8) n=9	11.9 (2.4) n=12				
26.3 (35.7) n=15	30.8 (38.1) n=15	-3.0 (-9.7 to 3.7)	0.345	-1.0 (-8.8 to 6.7)	0.780
8.3 (19.7) n=9	17.3 (35.7) n=13				
22.3 (27.3) n=15	22.7 (29.1) n=15	-0.7 (-7.5 to 6.0)	0.816	-2.9 (-12.9 to 7.1)	0.540
8.3 (15.2) n=9	14.6 (17.8) n=13				
14.0 (19.0) n=15	15.7 (22.3) n=15	-1.4 (-6.7 to 3.9)	0.575	-2.3 (-6.9 to 2.3)	0.303
6.7 (13.2) n=9	13.5 (20.1) n=13				

Table 1 Continued

		Mean (SD) Baseline period		
		T0	**T1**	**T2**
Secondary outcome Measures				
Echo intensity (sum z-scores)				
- Total	Intervention	NA	NA	18.9 (7.5) n=15
	Control	NA	NA	19.5 (6.7) n=10
- Lower limb	Intervention	NA	NA	11.0 (3.7) n=15
	Control	NA	NA	10.8 (3.1) n=10
- Upper limb	Intervention	NA	NA	7.9 (4.0) n=15
	Control	NA	NA	8.7 (4.3) n=10

*Between-group differences represent differences between the intervention and control group at T4, and these differences are adjusted for the clinical assessments (MFM, A6MCT, PEDI, timed tests, MRC, ROM or echo intensity) at T0-T2 as well as for corticosteroid use and ambulatory status at T2.
†Significant difference between the intervention and control group at the 0.05 level.
‡Non-parametric Wilcoxon Rank-sum tests were used; no 95%CI were, therefore, calculated.
Abbreviations: MFM, Motor Function Measure; A6MCT, Assisted Six-Minute Cycling Test; NA, Not assessed. PEDI, Pediatric Evaluation of Disability Inventory; 9HPT, Nine-hole peg test; MRC, Medical Research Council; ROM, passive joint range of motion; ext, extension; dfl, dorsiflexion

Primary outcome measures T4-T6

Eleven of the 16 participants that completed the intervention were followed up until T6. Five participants were lost to follow-up due to logistic reasons. The total MFM score had decreased on average by 2.5% (p=0.02) from T4 to T5 and by 5·7% (p=0.01) from T4 to T6. The D1 (-5.6%, p=0.02) and D2 (-6.6%, p=0.02) also showed a decline at T6, but the D3 (-4.3%, p=0.09) remained relatively stable. No significant changes in A6MCT scores were found for the legs (p=0.06) or the arms (p=0.71). (Table 2)

Secondary outcome measures

No significant group differences were found for the PEDI, timed tests, muscle strength, ROM and echo intensity at T4 (Table 1). However, 3 boys lost the ability to walk 10m and 2 boys lost the ability to rise from the floor in the control group, whereas in the intervention group none of the boys lost the ability to walk 10m and only 1 boy lost the ability to rise

Mean (SD) Training/ Control period		Secondary endpoint: Between-group differences at T3*		Primary endpoint: Between-group differences at T4*	
T3	**T4**	**Δ (95% CI)**	***p***	**Δ (95% CI)**	***p***
NA	18.8 (7.6) n=15	NA	NA	-1.3 (-5.3 to 2.6)	0.484
NA	21.3 (5.3) n=12				
NA	10.8 (3.7) n=15	NA	NA	-5.2 (-3.0 to 1.9)	0.660
NA	11.6 (3.1) n=12				
NA	8.0 (4.1) n=15	NA	NA	-0.5 (-2.8 to 1.7)	0.629
NA	9.7 (3.3) n=12				

from the floor during the training period. With respect to ROM, knee extension was more limited in the intervention group compared to the control group at baseline (p=0.03). However, ROM remained relatively stable in the intervention group during the training period, whereas (although not significantly) it increased from 7.1 to 17.3 for knee extension, from 7.9 to 14.6 for ankle dorsiflexion, and from 5.4 to 13.5 for elbow extension in the control group. This was the same for the echo intensity assessed with QMUS: although not significantly, the total score decreased with 0.1 z-score in the intervention group whereas it increased with 0.8 z-score in the control group.

Training effects in the control group after the waiting period

Nine of the 13 participants in the control group participated in the assisted bicycle training. One wheelchair-dependent participant had to be excluded prior to the start of the training because of a recent diagnosis of symptomatic cardiomyopathy. Three ambulant

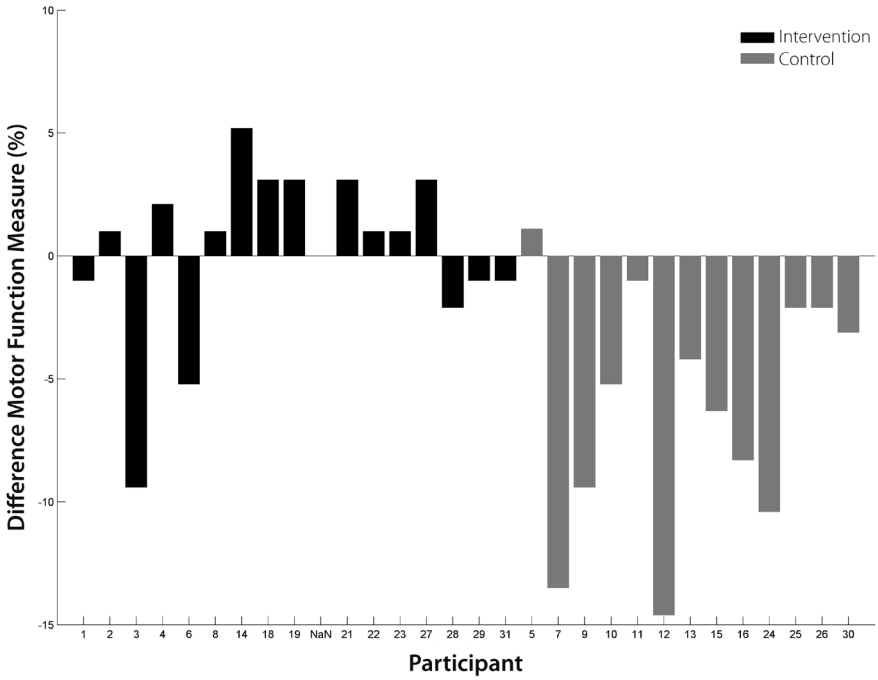

Figure 3 Individual differences in total MFM scores between T2 and T4.

Individual differences between the total MFM scores at T2 and T4 are shown for each participant in the intervention (black bars) and control (grey bars) group.
MFM, Motor Function Measure

boys had to be excluded within about 2 weeks after trying the training due to cognitive problems, a luxated patella, and a combination of fatigue and logistic problems. One participant fractured his femur during a fall at home unrelated to the training after 5 months of training. Eight out of nine participants therefore completed the 24-weeks training. The data of these remaining 8 control subjects showed an absence of functional decline during the training period comparable to the intervention group as assessed with the MFM, whereas no training effect was found for the A6MCT (Table 3).

Adverse events

No serious adverse events were observed or reported. During the training phase, postural adjustments were made in 3/24 participants who reported pain at the lateral side of the knee or foot due to an external rotation of the hip during training. In addition, 1 ambulant boy in the intervention group had an inversion trauma of his ankle after 12 weeks of training and subsequently stopped walking but he continued cycling. Another, wheel-

Table 2 Primary outcome measures at T4, T5 and T6 for the intervention group (n=11)*

	Mean (SD)				
	T4 (n=11)	**T5 (n=10)**	**T6 (n=11)**	**Mean difference between T4 and T5**	**Mean difference between T4 and T6**
MFM (%)					
- Total	69.6 (12.5)	67.1 (12.2)	64.6 (14.7)	-2.5 (2.7), $p = 0.02^{\dagger}$	-5.7 (5.2), $p = 0.01^{\dagger}$
- D1	32.6 (27.8)	30.8 (27.0)	27.0 (28.6)	-1.9 (7.4), $p = 0.43$	-5.6 (6.4), $p = 0.02^{\dagger}$
- D2	97.5 (2.6)	95.6 (4.9)	90.9 (9.0)	-1.7 (3.3), $p = 0.14$	-6.6 (8.1), $p = 0.02^{\dagger}$
- D3	93.5 (3.9)	91.9 (5.5)	89.2 (6.8)	-1.9 (4.0), $p = 0.17$	-4.3 (7.5), $p = 0.09$
A6MCT (Revolutions)					
- Legs	478.0 (93.3)	NA	447.3 (91/7)	NA	-30.7 (42.6), $p = 0.06$
- Arms	487.2 (97.0)	NA	494.8 (103.6)	NA	7.6 (58.0), $p = 0.71$

*Data of the participants who were lost to follow-up were excluded from the analysis.
†Significant difference at the $p < 0.05$ level.
Abbreviations: MFM, Motor Function Measure; A6MCT, Assisted Six-Minute Cycling Test; NA, Not assessed

Table 3 Training effect of the control group after the waiting period (n=8)*

	Mean (SD)				
	T4	**T5**	**T6**	**Mean difference between T4 and T5**	**Mean difference between T4 and T6**
MFM (%)					
- Total	66.0 (13.2)	68.2 (15.5)	68.2 (14.7)	2.2 (4.4), $p = 0.20$	2.2 (4.3), $p = 0.19$
- D1	34.0 (24.8)	35.3 (28.5)	34.0 (26.1)	1.3 (8.6), $p = 0.69$	0.0 (5.8), $p = 1.00$
- D2	87.8 (8.6)	89.6 (11.7)	89.6 (11.2)	2.1 (6.8), $p = 0.41$	1.7 (4.7), $p = 0.33$
- D3	88.1 (8.8)	92.3 (8.8)	95.2 (5.1)	4.2 (3.1), $p = 0.01^{\dagger}$	7.1 (5.1), $p = 0{\cdot}01^{\dagger}$
A6MCT (Revolutions)					
- Legs	470.8 (164.5)	NA	474.0 (161.5)	NA	3.3 (71.8), $p = 0.90$
- Arms	399.6 (94.2)	NA	409.3 (177.6)	NA	9.7 (124.7), $p = 0.84$

*Participants in the control group performed the same assisted bicycle training as the intervention group after a waiting period. Those who did not complete the training were excluded from the analysis.
†Significant difference at the $p < 0.05$ level.
Abbreviations: MFM, Motor Function Measure; A6MCT, Assisted Six-Minute Cycling Test; NA, Not assessed

chair-dependent, boy fractured his femur after 12 weeks of training. This boy continued cycling with his arms and he re-continued cycling with his legs after 3 weeks of immobilization. Injuries were unrelated to the training activities.

Discussion

The NUD study is the first trial in children with DMD testing the effects of assisted physical training on functional decline using a randomized controlled design. The results indicate that assisted bicycle training of the arms and the legs during 24 weeks significantly delays functional deterioration as tested with the MFM. The results also show that a low-intensity dynamic exercise training is feasible and safe even in boys who are in their late ambulatory phase or are wheelchair-dependent. If anything, the results contradict the current opinion that exercise training accelerates disease progression.

Previous studies have focused on resistance exercises and did not show clear training-induced benefits.[11-13] We found that assisted bicycle training prevented a 6.3% functional deterioration from the total MFM score (100%) during the intervention period as observed in the control group over a period of 24 weeks. This decline was larger than the 5.8% decrease of the MFM during 1 year reported previously by Vuillerot et al. (2010).[19] In that study participants were aged 6 to 32 years and also included individuals who were in their early ambulatory and late nonambulatory stage. The present study only included restricted walkers and boys who had recently become wheelchair-dependent who show a faster functional decline than ambulatory boys who are still in a relatively stable phase. Remarkably, the beneficial effects of training appeared to be strongest and most stable for the distal motor functions (D3 of the MFM). This is hardly surprising, as distal muscles are affected later than proximal muscles in DMD.[27] The beneficial effects on standing and transfers (D1 of the MFM) were smaller and disappeared fairly rapidly after cessation of the training. These observations support the notion that the adage "use it or lose it" is certainly applicable to boys with DMD.

In this study, the training-related preservation of standing, transfers, and distal motor functions was not paralleled by a preservation in axial motor functions (D2 of the MFM). This result was hardly surprising, as the bicycle training (where participants sat in a chair) placed no special demands on the axial muscle groups. We did not find an effect on overall endurance (as assessed with the A6MCT) either, which was most likely due to the low training intensity and the use of external electric support by the mobility trainer. The aim of the assisted bicycle training was to reduce the amount of secondary physical inactivity caused by disuse rather than to improve it by means of an endurance training with increasing demands. This probably also explains why no group differences were found with respect to secondary outcome measures such as strength. We believe that the assisted bicycle training may primarily preserve musculoskeletal flexibility and, thus, functional abilities, which is supported by the unchanged ROM of the ankle and elbow in the intervention group. It may also maintain cerebral representations of motor programs for both the arms and the legs, leading to preservation of functional skills. The training

does not impose serious demands on muscle strength or aerobic capacity. Cardiovascular endurance training aims at performing at 70-80% of the maximum heart rate, which might not be reached in our study. Aerobic training guided by heart rate is difficult in boys with DMD as their peak heart rate is hard to determine due to limited peripheral capacity [4]. In future studies, the mechanisms underlying the preservation of functional abilities in relation to training need to be further investigated.

This study is the first explorative RCT in children with muscle disease investigating the effects of physical training. It is also the first study that used assisted exercises instead of resistance training, which is often too hard to sustain for these children. We observed a high compliance level in most participants. This is encouraging as it suggests that this type of training can indeed be implemented in everyday life. However, a limitation is the fact that analysis was performed as treated, i.e. only those participants who adhered to the study protocol were included. We did not use an intention-to-treat analysis, because of the difficulty in handling missing data. A frequently used method to deal with missing data is carrying the last observation forward. This principle, however, is questionable in progressive muscle diseases. Nevertheless, missing data on the primary endpoints were limited to one drop-out in the intervention group. Furthermore, a limitation of this study is that randomization was incomplete. Two boys were directly allocated to the intervention group, because the study duration was ending. Another two boys were replaced to the control group after trying the intervention, because the training was unfeasible for them due to cognitive and motivation problems. A third limitation of this study is that we were not able to perform adequate test-retest reliability procedures on the primary outcome measure that showed a beneficial effect of training, i.e. the MFM. We opted for multiple baseline measurements with intervals of 4 weeks to control for initial severity and progression. This time interval might be too long to examine the variability of the MFM, as DMD progresses rapidly. Another limitation of this study is that both participants and the assessor were not blinded, since this was considered impossible. The assessor had to coach the participants and had to monitor for signs of overexertion during the training period. Participants and assessor were, however, uninformed about previous test results at each assessment. Nevertheless, memories from previous assessments cannot be entirely ruled out. However, the assessor was not able to remember the exact test scores of all 30 boys who were assessed with 4 to 12 weeks intervals. All assessments were performed by the same investigator to reduce inter-rater unreliability.

In conclusion, this study indicates that assisted bicycle training of the legs and arms is feasible and safe for both ambulant and wheelchair-dependent boys with DMD and that prolonged physical training can delay the secondary functional deterioration due to disuse in these children. Carrying out an intervention study in a progressive disorder such as DMD is however difficult due to the phenotypic variability, and further research is

required to support the results of this study and to enable clearly defined exercise protocols. Preferably, clinical trials should use stratified randomization to allocate their participants to either the intervention or control group according to the DMD phenotype: the common DMD form (ambulation is lost at a mean age of 9.3 years), the severe DMD form (ambulation is lost at a mean age of 7.1 years), or the milder DMD form (ambulation is lost at a mean age of 12.0 years).[29] If it is not possible to use stratified randomization, then researchers could limit the inclusion to one DMD subtype to reduce the phenotypic variability. To detect a decrease in disease progression, four to six times fewer boys are needed in a clinical trial including boys with the severe DMD form compared to a trial including boys with the common DMD form.[29] The cost-effectiveness of training should also be investigated, as it is hypothesized that the incorporation of assisted bicycle training in daily life can reduce the frequency of physiotherapy and can shift the role of the physiotherapist towards a coach and assessor in the management of DMD, at least during the relatively stable stages of the disease. It is expected that boys should start training early in their disease course, as disuse could start at a young age in DMD.

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Contributors

IJMG and NA handled funding and designed the first draft of the study protocol. ACHG provided methodological advice. All authors developed and wrote the final study protocol. MJ coached the participants during the training and performed the clinical assessments. Data analyses and interpretations were performed by all authors and were supervised by two independent statisticians. IJMG was responsible for supervision of the project. All authors contributed to writing the manuscript and approved the final version.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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References

1. Emery AE. Population frequencies of inherited neuromuscular diseases--a world survey. *Neuromuscul Disord* 1991;1:19-29.
2. Bach JR, Martinez D. Duchenne muscular dystrophy: continuous noninvasive ventilatory support prolongs survival. *Respir Care* 2011;56:744-750.
3. Eagle M, Bourke J, Bullock R et al. Managing Duchenne muscular dystrophy--the additive effect of spinal surgery and home nocturnal ventilation in improving survival. *Neuromuscul Disord* 2007;17:470-475.
4. Bar-Or O, Rowland TW. Neuromuscular and musculoskeletal diseseases. In: Bar-Or O, Rowland TW, editors. *Pediatric exercise medicine. From physiologic principles to health care application.* United States of America: Human Kinetics; 2004. p 269-321.
5. McDonald CM. Physical activity, health impairments, and disability in neuromuscular disease. *Am J Phys Med Rehabil* 2002;81:S108-S120.
6. McDonald CM, Abresch RT, Carter GT et al. Profiles of neuromuscular diseases. Duchenne muscular dystrophy. *Am J Phys Med Rehabil* 1995;74:S70-S92.
7. Petrof BJ. The molec*ular basis of activity-induced muscle injury in Duchenne muscular dystrophy.* Mol Cell Biochem 1998;179:111-123.
8. Lim JH, Kim DY, Bang MS. Effects of exercise and steroid on skeletal muscle apoptosis in the mdx mouse. *Muscle Nerve* 2004;30:456-462.
9. Bushby K, Finkel R, Birnkrant DJ et al. Diagnosis and management of Duchenne muscular dystrophy, part 2: implementation of multidisciplinary care. *Lancet Neurol* 2010;9:177-189.
10. Voet NB, van der Kooi EL, Riphagen II, Lindeman E, van Engelen BG, Geurts AC. Strength training and aerobic exercise training for muscle disease. *Cochrane Database Syst Rev* 2010:CD003907.
11. Vignos PJ, Jr., Watkins MP. The effect of exercise in muscular dystrophy. *JAMA* 1966;197:843-848.
12. de Lateur BJ, Giaconi RM. Effect on maximal strength of submaximal exercise in Duchenne muscular dystrophy. *Am J Phys Med* 1979;58:26-36.
13. Scott OM, Hyde SA, Goddard C, Jones R, Dubowitz V. Effect of exercise in Duchenne muscular dystrophy. *Physiotherapy* 1981;67:174-176.
14. Hayes A, Williams DA. Beneficial effects of voluntary wheel running on the properties of dystrophic mouse muscle. *J Appl Physiol* 1996;80:670-679.
15. Call JA, McKeehen JN, Novotny SA, Lowe DA. Progressive resistance voluntary wheel running in the mdx mouse. *Muscle Nerve* 2010;42:871-880.
16. Sveen ML, Jeppesen TD, Hauerslev S, Kober L, Krag TO, Vissing J. Endurance training improves fitness and strength in patients with Becker muscular dystrophy. *Brain* 2008;131:2824-2831.
17. Jansen M, de Groot I, van Alfen N, Geurts AC. Physical training in boys with Duchenne Muscular Dystrophy: the protocol of the No Use is Disuse study. *BMC Pediatr* 2010;10:55.
18. Bar-Or O, Rowland TW. Procedure for exercise testing in children. In: Bar-Or O, Rowland TW, editors. *Pediatric exercise medicine. From physiologic principles to health care application.* United States of America: Human Kinetics; 2004. p 343-365.
19. Vuillerot C, Girardot F, Payan C et al. Monitoring changes and predicting loss of ambulation in Duchenne muscular dystrophy with the Motor Function Measure. *Dev Med Child Neurol* 2010;52:60-65.
20. Robertson RJ, Goss FL, Boer NF et al. Children's OMNI scale of perceived exertion: mixed gender and race validation. *Med Sci Sports Exerc* 2000;32:452-458.
21. Jansen M, De Jong M, Coes HM, Eggermont F, Van Alfen N, De Groot IJ. The assisted 6-minute cycling test to assess endurance in children with a neuromuscular disorder. *Muscle Nerve* 2012;46:520-530.
22. Berard C, Payan C, Hodgkinson I, Fermanian J. A motor function measure for neuromuscular diseases. Construction and validation study. *Neuromuscul Disord* 2005;15:463-470.
23. Custers JW, Wassenberg-Severijnen JE, van der Net J, Vermeer A, Hart HT, Helders PJ. Dutch adaptation and content validity of the 'Pediatric Evaluation Of Disability Inventory (PEDI)'. *Disabil Rehabil* 2002;24:250-258.
24. Medical Research Council of the United Kingdom. *Aids to examination of the peripheral nervous system: Memorandum No 45.* Palo Alto, CA: Pedragon House; 1978.

25. Norkin CC, White DJ. *Measurement of Joint Motion: a guide to goniometry.* 3rd ed. Philadelphia, US: Davis Company; 2003.
26. Scholten RR, Pillen S, Verrips A, Zwarts MJ. Quantitative ultrasonography of skeletal muscles in children: normal values. *Muscle Nerve* 2003;27:693-698.
27. Jansen M, van Alfen N, Nijhuis van der Sanden MW, van Dijk JP, Pillen S, de Groot IJ. Quantitative muscle ultrasound is a promising longitudinal follow-up tool in Duchenne muscular dystrophy. *Neuromuscul Disord* 2012;22:306-317.
28. Cohen J. *Statistical power analysis for the behavioral sciences.* 2nd ed. New Jersey, US: Lawrence Erlbauw Associates; 1977.
29. Humbertclaude V, Hamroun D, Bezzou K et al. Motor and respiratory heterogeneity in Duchenne patients: implication for clinical trials. E*uropean journal of paediatric neurology : EJPN : official journal of the European Paediatric Neurology Society* 2012;16:149-160.

7

Upper limb training with dynamic arm support in boys with Duchenne muscular dystrophy: a feasibility study

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Submitted

Abstract

Purpose: Patients with Duchenne muscular dystrophy (DMD) develop progressive loss of arm function, which severely limits the patient's activities of daily living and independence. Although regular moderate-intensity activities are recommended to prevent secondary complications of physical inactivity, conventional resistance exercises are often too strenuous for patients with DMD. We conducted a feasibility study with a multiple N=1 design to investigate the feasibility and safety of upper limb training with dynamic arm support (DAS) for DMD patients who are unable to lift their arms against gravity.

Method: Eight participants (age 12-20 years) performed reaching movements with their non-dominant arm for 24 weeks by playing a virtual reality computer game and performing activities of daily living while using DAS. The dominant (untrained) arm of each participant served as a reference.

Results: Six of the eight participants completed the entire training program (the other two participants discontinued training due to practical reasons). No serious adverse events were reported. Among the four participants who completed the program, the trained arm retained more motor function than the untrained arm.

Conclusions: The findings indicate that boys with DMD who have impaired arm function can train their upper limb with DAS to improve motor function.

Introduction

Duchenne muscular dystrophy(DMD) is the most common childhood neuromuscular disorder (NMD), affecting 1 in 3500 live male births.[1] DMD is caused by a loss of the dystrophin protein, which causes progressive muscle fibrosis, loss of muscle strength and loss of muscle function.[2] Although there is currently no cure, improved symptomatic treatments have increased the mean life expectancy of patients with DMD to the early thirties.[3] As a result of this increased life expectancy, there is now a growing population of older, wheelchair-dependent patients with severely impaired arm function and associated limitations in activities of daily living (ADLs) such as grooming and dressing.

The decline in arm function in patients with DMD is primarily a result of the disease; wasting of the shoulder muscles causes an inability to lift the arms by the age of 13-15 years.[4] This inability to lift the arms limits the patients to reaching forward and sideward only. This decline in arm function can also be accelerated by environmental and behavioral factors. For example, using an electric wheelchair with a top blade and central operating joystick allows the patient to function within the confines of the wheelchair only. This can cause a discrepancy between the patient's true capacity and their performance (i.e., disuse), which can reduce the level of physical activity for the upper limb and reduce arm function even further. The negative consequence of disuse is underscored by the increasing difference in strength and motor function between the dominant and non-dominant hands over time—specifically, the non-dominant hand is used less and becomes less useful than the dominant hand.[5,6] From this point of view, the adage "use it or lose it" clearly applies to patients with DMD.

The current international guidelines regarding physical activity for DMD patients recommend regular gentle, functional activity in order to avoid secondary complications due to physical inactivity.[7] However, these recommendations are based on only a limited amount of evidence. Although three non-controlled trials found that low-resistance exercises were safe for ambulatory boys with DMD, these exercises had only a limited positive effect on muscle strength and function.[8-10] Another limitation of resistance exercises is that they are often impractical for wheelchair-dependent boys, as even the lowest resistance level can be too heavy to sustain or can rapidly exhaust their weak muscles.[11] Finally, strenuous high-resistance exercises and eccentric exercises can cause myofibrillar disruption in patients with DMD.[12]

A recent randomized controlled trial found that assisted bicycle training is both feasible and safe for ambulatory and recently wheelchair-dependent patients with DMD.[13] Moreover, this training program prevented 6.3% of the functional deterioration that occurred without this intervention. In this study, boys trained their legs and arms using a

mobility trainer with electrical motor support. Although assisted training provides training opportunities to patients with DMD who have been confined to a wheelchair for several years, arm cranking may not be possible for patients who cannot lift their arms. We hypothesized that training with dynamic arm support (DAS) may be a viable solution for these patients. DAS provides horizontal and vertical external mechanical support for weak muscles via non-powered (e.g., counterweights) or powered components and increases the active range of motion for joints.[14]

The aim of this study was to investigate whether DAS-assisted upper limb training is feasible and safe for wheelchair-dependent patients with DMD who are unable to lift their arms against gravity. We expected that patients would be able to practice reaching movements, which have high priority in patients with a neuromuscular disorder[15], with a DAS without causing harm. Because no previously published study has used DAS to train upper limb function in patients with DMD, and because the safety is a concern with exercise in DMD patients, we elected to perform a feasibility study with a multiple N=1 design. The goal of this study was to determine whether this type of assisted training is safe and can help patients with DMD. In addition, we measured—albeit to a limited extent—the effect of DAS-assisted upper limb training on motor function.

Methods

Design

This study was a feasibility study with a multiple N=1 design. Each participant acted as his own internal control (i.e., the participant's untrained arm served as the reference for the trained arm). A detailed description of the study protocol has been published previously.[16] The study was approved by the regional Medical Ethics Committee. The participants and/or legal guardians provided written informed consent. The study was registered with the Netherlands Trial Register (trial number 1631).

Participants

Wheelchair-dependent boys with DMD who were unable to touch the top of their head with at least one hand but were still able to use their hands for tabletop activities such as writing were eligible for this study. At the start of recruitment, an estimated 120 boys with DMD within the Netherlands were in this stage of the disease. Boys were excluded if they were unable to touch their nose while using the DAS, as this movement was required for several daily activities, including feeding, which was part of the intervention. In addition, boys who had previously used DAS were excluded. Boys were recruited from the Dutch Duchenne Parent Project database. Recruitment started 1 January, 2009 and ended 31 December 2009.

Procedure

After an eight-week period (T0-T2; see Figure 1) in which baseline information was collected regarding the stability of the patient's disease course, each participant was provided with a powered or non-powered DAS device (Dynamic Arm Support Top/Help, non-powered or powered versions, Focal Meditech BV, Tilburg, the Netherlands) for their non-dominant arm. At baseline (T2), the participants began a 24-week training program for their non-dominant arm using DAS. Participants performed forward and sideward reaching movements by playing a computer game and by performing regular daily activities such as eating. For this study, we chose to train the non-dominant arm, as this arm is often used less than the dominant arm in daily activities and thus may experience more disuse than the dominant arm; thus, we expected any potential training effects to be more pronounced. Efficacy was assessed after 12 weeks (T3) and at the primary endpoint of 24 weeks of training (T4). A final follow-up assessment was performed 12 weeks after the end of the training (T5). For the complete timeline of the study, see figure 1.

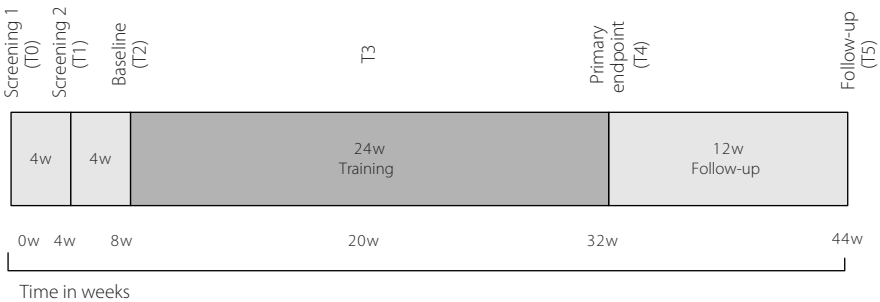

Figure 1 Study design.

The participants were provided with a dynamic arm support at T2.
W, weeks

Dynamic arm support (DAS)

The DAS Top/Help device originally been developed to be an assistive device. It has a weight-bearing construction mounted beneath the user's arm (see Chapter 5, Figure 5). The device aims to provide an active range of motion-assisting movements in the horizontal and vertical planes. The "Top" part of the DAS device consists of an axis with several rotation points, a forearm fitting, and a connection to the wheelchair. The "Help" part of the DAS device uses a mechanical spring mechanism (mechanical arm support) or an electric actuator (electric arm support) to facilitate vertical displacements. Participants who were unable to touch their nose with the help of a mechanical arm support received an electric arm support.

Computer-assisted training

The participants practiced DAS-assisted forward and sideward reaching movements five days per week by playing the virtual-reality computer game "FurballHunt"[1] without becoming overexerted (as assessed using the OMNI scale).FurballHunt is controlled by motion capture technology using a webcam to detect gross arm movements.[17]

Functional training

The participants were also instructed to eat at least two meals per week while using the DAS device. In addition, they were instructed to use DAS as much as possible while performing daily activities that involved reaching movements of the arms (for example, turning on the lights).

Feasibility and safety assessments

We monitored whether participants were able to complete the entire training program in accordance with the protocol, without any signs of overexertion. This was assessed via a questionnaire completed once every two weeks and home visits by the primary investigator (MJ) after 2 and 12 weeks of training. The participants recorded their training sessions in a journal and kept written records of the primary activities that they performed with the DAS device. The questionnaire regarding overexertion focused on excessive muscle pain, a feeling of severe discomfort feeling, and exhaustion (OMNI score[18] >6).

Efficacy assessments

All assessments were performed by the primary investigator (MJ) either at the hospital (for assessments in T2 and T4) or at the participant's home (for the other assessments). Both the dominant (untrained) and non-dominant (trained) arms were assessed. The non-dominant arm was assessed without DAS; in addition, to gain information regarding the benefits of training with the device, the non-dominant arm was assisted with DAS at baseline (T2).

The primary outcome was the Action Research Arm Test (ARAT), a standardized tool for assessing arm motor function and capacity in stroke patients.[19,20] At the time of the study, no validated arm motor function test for assessing boys with DMD was available. We calculated the total ARAT score (range 0-57) as well as the score of subscale D (gross arm movements, range 0-9), with a higher score indicating better function. An adjustable-height table is needed to properly administer the test execution, and because such a stable was usually not available in the participant's home, the total ARAT was measures at T0, T2 and T4.

1 "Furballhunt is developed by Roessingh Research and Development.

The secondary outcome measures were Dimension 3 (distal motor function) of the Motor Function Measure (MFM D3) to assess motor function[21], the Nine-Hole Peg Test (9HPT) to assess finger dexterity[22], and the Jebsen Taylor Hand Function Test (referred to here as the Jebsen Test) to assess hand function[23]; these measured were collected at T2 and T4. Although the 9HPT and Jebsten Test assess primarily hand function, both tests force participants to lift and/or reach with their arms. At the end of the training period (T4), the participants also received a questionnaire designed to collect information regarding their DAS training experiences.

Statistical analysis

This was a feasibility study with a multiple N=1 design; therefore, the feasibility, safety and effectiveness of the DAS training program are described separately for each participant. The effectiveness of training was measured by calculating the change in the untrained and trained arms from T2 to T4; assessment T2 was chosen as our baseline value because T2 was closest to the time in which the participants received their DAS device and began training. In addition, the immediate effectiveness of using the DAS was assessed by comparing the results of the non-dominant arm without and with DAS at baseline (T2).

Results

Participants

Thirteen boys were initially assessed for eligibility. Five were excluded because they were unable to touch their nose even with the use of DAS. Thus, eight boys (age 12-20 years, with wheelchair dependence for 3-10 years) were first assessed for their baseline parameters and then provided with a DAS, after which they began training. The participants' score on the Brooke scale[24] ranged from 3 (cannot raise hands above the head, but can raise an 8-ounce glass of water to the mouth) to 5 (able to use the hands for some daily activities only). A full overview of the participants' characteristics is presented in table 1.

Effectiveness of the Dynamic Arm Support (DAS)

All eight participants were provided with a DAS device for their non-dominant (left) arm. Six participants received an electrical arm support, and two participants (who had only been fully wheelchair-dependent for 3 to 3.5 years) received a mechanical arm support (Table 1).

At baseline (T2), when using the DAS, the total ARAT score increased by 11-17 points for four of the eight participants, whereas the total ARAT score was either unchanged or decreased by up to 14 points for the other four participants. We found that the configuration of the ARAT testing board interfered with the use of the DAS device. This

Table 1 Characteristics of the participants (N=8)

ID	Age (years)	Years wheelchair-dependent	Cortico-steroids	Brooke (range 0-6)	DAS	Compliance
1	12.1	3	Yes	3	Mechanical	5x p/w
2	15.4	5	Yes	5	Electrical	4x p/w
3	12.1	4	No	4	Electrical	Inadequate*
4	20.0	7	No	5	Electrical	3x p/w
5	14.1	3.5	Yes	3	Mechanical	4x p/w
6	15.9	7	Yes	3	Electrical	3x p/w
7	18.9	10	No	5	Electrical	Inadequate†
8	15.0	4	Yes	3	Electrical	Inadequate‡

*Social support was limited, and the computer broke during the study.
† Unexpected trip for 3 months; lost to follow-up.
‡ New wheelchair without DAS; lost to follow-up.
DAS, Dynamic Arm Support; p/w, per week

effect was most pronounced for the participants who scored relatively high (>20) without using the DAS. With respect to subscale D, this score increased by 1-4 points for seven of the eight participants. The score decreased by 2 points for the eighth participant; this participant was unable to touch the top of his head with DAS, although he was able to touch the top of his head without DAS. Thus, the DAS device hindered this movement for this participant. The scores for all eight participants are summarized in table 2.

Similar to changes in the ARAT scores, the baseline (T2) MFM D3 and 9HPT scores were also improved with DAS (Table 2). On the other hand, the Jebsen Test scores did not improve with the DAS (with the exception of one item—the time required to pick up and drop small common objects) (Table 2). As occurred when performing the ARAT, the Jebsen Test board interfered with the DAS device, and maximum elbow extension was restricted by the mechanical construction of the device. This effect was most pronounced for the items that required reaching movements (for example, turning cards and moving objects).

Feasibility and safety of the DAS-assisted training program

Of the eight participants who began training, two did not complete the program; one boy had an unexpected three-month stay abroad, and the other boy obtained a new wheelchair (without the DAS installed). The remaining six participants finished the training program and practiced computer-assisted training 3-5 times per week. One boy had fewer training sessions because his computer broke, and he was unable to play during the first eight weeks. In addition to the computer-assisted training sessions, use of the DAS

Table 2 Function test scores with and without DAS at baseline (T2)

	ARAT		MFM, D3 (%)	9HPT (sec)	Jebsen (sec)					
	Total (range 0-57)	Scale D (range 0-9)			J1	J2	J3	J4	J5	J6
ID 1										
Without	19	3	66.7	28.7	ND	ND	ND	ND	ND	ND
With	31	7	71.4	32.1	ND	ND	ND	ND	ND	ND
Difference	12	4	4.8	3.3	NA	NA	NA	NA	NA	NA
ID 2										
Without	9	3	66.7	49.1	8.4	U	U	6.8	13.2	U
With	20	4	66.7	49.8	21.6	28.7	67.0	6.2	12.2	U
Difference	11	1	0	0.7	13.2	NA	NA	-0.6	-1.0	NA
ID 3										
Without	24	3	71.4	40.6	13.6	20.2	41.3	7.5	5.3	9.0
With	23	4	76.2	32.0	23.6	18.6	44.2	6.7	10.9	15.1
Difference	-1	1	4.8	-8.6	10.0	-1.7	2.9	-0.8	5.6	6.2
ID 4										
Without	21	4	66.7	83.0	42.6	54.6	37.9	7.8	26.7	39.8
With	21	5	61.9	47.6	22.7	21.8	24.3	6.3	23.5	33.5
Difference	0	1	-4.8	35.4	-19.9	-32.9	-13.7	-1.4	-3.3	-6.3
ID 5										
Without	54	7	66.7	21.8	5.7	8.2	24.3	3.8	4.9	8.3
With	40	5	76.2	25.1	7.3	9.2	24.4	6.9	4.4	7.5
Difference	-14	-2	9.5	3.2	1.6	1.0	0.1	3.1	-0.6	-0/8
ID 6										
Without	20	4	66.7	35.6	8.4	15.8	47.0	4.5	8.4	20.0
With	19	4	76.2	41.7	14.2	14.5	U	5.2	6.7	21.3
Difference	-1	0	9.5	6.0	5.8	-1.3	NA	0.7	-1.7	1.3
ID 7										
Without	2	0	57.1	164	U	U	79.0	U	U	U
With	19	3	62.0	ND	ND	ND	ND	ND	ND	ND
Difference	17	3	4.8	NA	NA	NA	NA	NA	NA	NA
ID 8										
Without	8	0	62.0	41.3	8.5	28.5	35.4	4.1	5.5	8.7
With	19	4	66.7	36.3	19.7	23.9	80.0	5.3	11.7	16.6
Difference	11	4	4.7	-5.0	11.1	-4.6	54.6	1.2	6.2	7.9

ARAT, Action Research Arm Test; MFM, Motor Function Measure; 9HPT, Nine-Hole Peg Test; Jebsen, Jebsen Taylor Hand Function Test; J1, Turning cards; J2, Small objects; J3, Feeding; J4, Stacking; J5, Light objects; J6, Heavy objects; ND, Not determined; U, Unable to perform; NA, Not applicable

ranged from seldom to 2 hours a day for the following activities: eating and drinking (7/8 participants), horizontal and vertical reaching activities such as turning on the computer or a light (1 participant), and using a pin-card (1 participant). The use of DAS by the participants is summarized in table 1.

No signs of serious overexertion were reported by the participants. One participant reported pain in his shoulder during the training; he reported that his shoulder was painful when he reached forward and moved his arm above 90º of anteflexion. When we adjusted this participant's training program (the participant was instructed not to raise his arm above shoulder height), the pain disappeared and he completed the training program.

Effects of the DAS-assisted training program

Of the six participants who completed the program, four had a larger decrease in ARAT score in the untrained arm compared to the trained arm; for the other two participants, the untrained arm scores decreased slightly less than the trained arm after 24 weeks of training (T4). This more pronounced decrease in the untrained arm was particularly pronounced for the participant who was still able to raise a glass to his mouth at baseline; similar results were obtained for subscale D of the ARAT for this participant. The remaining five participants had no differences between their trained and untrained arm with respect to subscale D at T3 or T4. These scores are summarized in table 3.

With respect to the secondary outcomes, none of the participants had a decrease in MFM D3 score for the trained arm after 24 weeks of training (T4). For the untrained arm, however, four participants had a decrease in MFM D3 score (the change in score for these four participants ranged from -4.8 to -9.5%); the other two participants had no change. The 9HPT score decreased for the trained arm for two participants and decreased for the untrained arm for three participants. Finally, the Jebsen Test revealed no notable differences between the trained and untrained arms, with the exception of the item in which the participants had to pick up and drop small common objects—at T4, two participants lost the ability to pick up the objects with their untrained arm, although they retained the ability with their trained arm. The secondary outcomes are summarized in table 3.

Follow-up 12 weeks after the end of the training

Of the six participants who completed the training program, five participants were followed until 12 weeks after the end of the training program (T5), and two of the participants had continued to use the DAS device, but without specific training. The sixth participant was lost to follow-up due to a scoliosis surgery. Twelve weeks after training, Dimension 3 of the ARAT had not decreased for any of the participants for either the trained or untrained arm. Similar results were obtained for the MFM D3 and 9HPT tests at T5. The follow-up data are summarized in table 4.

Table 3 Effects of 24 weeks of training with dynamic arm support (N=6)

	ARAT		MFM, D3 (%)	9HPT (sec)	Jebsen (sec)					
	Total	**Scale D**			**J1**	**J2**	**J3**	**J4**	**J5**	**J6**
ID 1										
Trained	6	1	4.8	5.6	ND T2	ND T2	ND T2	ND T2	ND T2	ND T2
Untrained	-16	-4	-4.8	2.4	ND T4	ND T4	ND T4	ND T4	ND T4	ND T4
ID 2										
Trained	-2	-2	0	0.4	U T4	U T2/T4	U T2/T4	-1.8	U T4	U T2/T4
Untrained	-4	-2	-9.5	-17.0	2.5	10.3	U T2/T4	-1.7	-2.3	U T2/T4
ID 3										
Trained	-13	-2	0	-7.2	-3.3	0.8	U T4	-1.7	5.4	2.3
Untrained	-21	-2	-4.8	1.1	-0.7	U T4	U T4	-3.3	1.3	2.0
ID 4										
Trained	0	0	0	-8.0	19.9	-21.6	U T4	-0.7	U T4	U T4
Untrained	-9	0	0	-4	-4.9	U T4	U T4	-2.6	U T4	-10.7
ID 5										
Trained	-14	-1	4.8	-4.8	-0.6	-1.7	-7.5	-2.0	-0.4	-2.8
Untrained	-8	-1	0	-10	0.8	-4.0	-1.9	-4.4	-1.6	-1.1
ID 6										
Trained	-2	0	0	-4.1	-0.6	-0.5	9.5	0.8	0.6	U T4
Untrained	2	0	-4.8	14.4	-4.8	1.3	U T4	0.8	-5.1	U T4

Data are individual differences in scores between T2 (start of training) and T4 (primary endpoint) for the trained and untrained arms.
ARAT, Action Research Arm Test; MFM, Motor Function Measure; 9HPT, Nine-Hole Peg Test; Jebsen, Jebsen Taylor Hand Function Test; J1, Turning cards; J2, Small objects; J3, Feeding; J4, Stacking; J5, Light objects; J6, Heavy objects; ND, Not determined at the indicated assessment(s); U, Unable to perform at the indicated assessment(s)

Participants' experiences

The participants reported that they found DAS useful for the following activities and movements: lifting their arm, scratching their face, moving their arm beyond the tray of the wheelchair, and feeding themselves. However, the DAS limited their ability to reaching down and to open a door. In addition, eating with a fork or spoon was challenging, as the DAS device did not allow the full range of supination movements. Sitting at a table while using the DAS device was also problematic because of interference between the device and the table. Nevertheless, six of the eight participants requested to keep the DAS at the end of the study; these six participants were the patients who were unable to lift their arms against gravity without DAS and had received electrical support. Although all of the

Table 4 Follow-up after the end of the training

Test/ ID	ARAT		MFM, D3 (%)	9HPT (sec)
	Total	Scale D		
ID 1				
Trained	ND	0	0	-3.1
Untrained	ND	0	4.8	-4.2
ID 2				
Trained	ND	0	-9.5	11.5
Untrained	ND	0	4.8	20.6
ID 3				
Trained	ND	0	0	12.1
Untrained	ND	0	9.5	-2.1
ID 4				
Trained	ND	M	0	-13.0
Untrained	ND	0	-4.8	3.0
ID 5				
Trained	ND	0	4.7	4.8
Untrained	ND	0	0	6.0
ID 6				
Trained	ND	M	M	M
Untrained	ND	M	M	M

Data are individual changes between T4 (primary endpoint) and T5 (follow-up) for the trained and the untrained arm. ARAT, Action Research Arm Test; MFM, Motor Function Measure; 9HPT, Nine-Hole Pegtest; M, Missing; ND, Not determined (assessment was conducted at home)

participants liked the computer-assisted training at the start of the training, none of the participants found the game attractive after 24 weeks of training.

Case narratives

ID 1: Participant 1 was a 12-year-old boy who had been fully wheelchair-dependent for three years at the start of the study. He was still able to raise a glass of water to his mouth, and he was provided with a mechanical DAS device. The DAS increased his arm and hand motor function as assessed with the ARAT, MFM and 9HPT tests. He completed the entire training program with good compliance (i.e., he performed the computer-assisted training an average of 4-5 times per week). He reported that the DAS device hindered his ability to reaching the table surface because of interference between the device and the table. Opening a door and reaching down were difficult as well because his arm fell out of the forearm support. Nevertheless, he ate once a week with DAS, and he reported that scratching his face and lifting objects (such as a glass) were easier with DAS. After 24 weeks

of training, his ARAT and MFM D3 scores were either unchanged or increased; in contrast, the scores for his untrained arm had decreased considerably, particularly with respect to the ARAT.

ID 2: Participant 2 was 15-year-old boy who had been confined to an electric wheelchair for five years at the start of the study. This patient only had function remaining in his hands and was provided with an electric DAS device. The device increased his gross arm movements as assessed with the ARAT, and it allowed him to complete the feeding and lifting small objects tasks of the Jebsen Test (which were not possible without support). He completed the training program and performed the computer game four times per week. In additionally, he ate once every two weeks with his DAS, but reported that it was difficult to eat with his non-dominant hand (the trained arm). After 24 weeks of training, his MFM D3 score decreased for the untrained arm, but not the trained arm. At the end of the study, the participant requested to keep the DAS device to use for his dominant arm, but he did not wish to continue using the computer game.

ID 3: Participant 3 was a 12-year-old boy who had been fully wheelchair-dependent for four years at the start of the study. He was able to raise his hand—but not a glass—to his mouth. He was provided with an electric arm support, which increased his hand function as assessed with the MFM and 9HPT, but hindered his gross arm movements. Thus, the DAS device interfered with the board required to perform the ARAT and 9HPT tests. In addition, the DAS limited his elbow extension (which was needed to perform the reaching tasks of the Jebsen Test), thereby increased the time needed to complete the tasks. The participant completed the training program, but with moderate compliance during the first eight weeks of training due to technical problems with his computer. Nevertheless, he ate once a week with the DAS—which was difficult for him—with his non-dominant (trained) hand. The participant lost the ability to perform the feeding and lifting small objects tasks of the Jebsen Test with his untrained arm, whereas he was still able to complete these tasks test with his trained arm.

ID 4: Participant 4 was a 20–year old male (the oldest participant) who had been wheelchair-dependent for seven years at the start of the study. He was unable to bring his hands to his mouth. The DAS helped him lift his hand, which was reflected in the decreased time needed to perform the Jebsen Test tasks "Turning cards", "Feeding", and "Small objects". The participant used the DAS approximately two hours per day for eating, drinking, and other reaching and lifting activities such as turning on the light. We found no clear differences in arm function between the trained and untrained arms after 24 weeks of training; however, his scores were already low at baseline.

ID 5: Participant 5 was a 15-year-old boy who had been wheelchair-dependent for 3.5 years at the start of the study. He was still able to lift a glass to his mouth. He was provided with a mechanical arm support, which limited his gross arm movements. In fact, his own compensatory movements were more helpful in terms of performing the clinical assessments than the DAS device. The participant performed the computer-assisted training an average of four times per week, but he rarely used the DAS to perform functional activities. After 24 weeks of training, there were no clear differences between his trained and untrained arms with respect to change in arm function.

ID 6: Participant 6 was nearly 16 years of age at the start of the study. Although he was still able to lift a glass to his mouth, this effort exhausted him, and he was provided with an electric arm support. Although the DAS device hindered his gross arm movements, the device made eating easier, and he used the DAS device every day for eating. Furthermore, he performed the computer-assisted training with DAS approximately three times per week. After 24 weeks of training, he lost the ability to perform the feeding task of the Jebsen Test with his untrained arm, whereas he was still able to perform this task with his trained arm.

ID 7: Participant 7 was an 18–year-old boy who had been confined to a wheelchair for ten years at the start of the study. He had useful hand function, but he was unable to lift his hand to his mouth. He was provided with an electric arm support that was on a moveable frame, as the corridor in his house was too small to enable him to maneuver his wheelchair when the device was attached. The DAS increased his arm function considerable as assessed with the ARAT. The participant did not complete the training program, and he was lost to follow-up because he went on an unexpected trip for three months.

ID 8: Participant 8 was a 15-year-old boy who had been fully wheelchair-dependent for four years at the start of the study. He was able to lift his hand—but not a glass—to his mouth. He was provided with an electric DAS, which increased his gross arm movements. However, he did not use the device for daily activities primarily because the participant did not want to use the DAS at school (he feared that his peers would think it was not "cool"). He did not complete the training program because halfway through the study, he received a new wheelchair that was not equipped with a DAS device (i.e., we would have needed to transfer to the old wheelchair to use the DAS). Surprising, the participant requested to keep the DAS at the end of the study, stating that the device allowed him to use an Automated Teller Machine (ATM).

Discussion

This feasibility study revealed that patients with DMD who have impaired arm function can safely participate in assisted training. Thus, patients who are unable to lift their arms against gravity are able to practice reaching forward and sideward movements by playing a virtual reality computer game while using a DAS device. Furthermore, we found indications that assisted training may slow the loss of arm motor functions. Because delaying loss of motor function is important to boys with DMD who wish to maintain their activities of daily living (ADLs), our findings indicate that upper limb training with assistance warrants further research.

The Top/Help DAS device was moderately effective for our training purposes; DAS increased gross arm motor functions (such as the ability to lift the hand to the mouth) in seven out of eight participants and allowed the participants to train without the help of a physiotherapist. This finding is in agreement with previous studies that reported that several ADLs can be improved with DAS.[25,26] The only participant who had a decrease in gross arm motor function after using the DAS was still able to lift a glass to his mouth without the device. The DAS prevented him from placing his hand on his head, an action that he could perform using his own compensatory strategies (i.e., without DAS). This is similar to previous reports that also showed that stronger children prefer their own compensatory strategies to overcome their limitation.[14,27] In addition, hand function—which is associated with muscle strength and range of motion[28]—did not improve using DAS, with the exception of the task in which the participant was required to pick up and drop small objects. This lack of improved hand function is in contrast to previous findings[14], which can be explained by that fact that the DAS device interfered with the equipment required for the hand function test. In addition, some hand functions (such as picking up beans with a spoon) were difficult to perform with the DAS, as the participants found it difficult to stabilize their hand with the device. Finally, the DAS prevented full elbow extension and supination, which are needed to perform the reaching tasks.

The assisted training program was determined to be feasible for six of the eight participants. Two of the participants did not complete the entire training program for practical reasons. The remaining six participants completed the training with moderate compliance (they performed the computer-assisted training 3-5 times per week). Only two of the participants were fully compliant with the functional training program and ate at least one meal twice a week with the DAS device. The other participants used the DAS less frequently, primarily because they found it difficult to use their non-dominant (i.e., the trained) arm to perform ADLs. In addition, the participants who were still able to lift a glass to their mouth without DAS preferred to use their own compensatory strategies, which is similar to previous reports. Compliance might be improved by providing more stimulating computer games and/or by providing a DAS that assists the arm through its entire range

of motion. Future clinical trials on functional arm training should also consider to train the dominant arm rather than the non-dominant arm, since it is often not appropriate to train this arm.

Four of the six participants who completed the program had more deterioration of arm motor functions for the untrained arm than for the trained arm as assessed with the ARAT. In addition, none of the participants showed a decline on the distal motor function dimension of the MFM with the trained arm, whereas the untrained arm deteriorated in four participants and remained stable in the other two participants. Previous studies using gentle resistance exercises in DMD patients also found no disease acceleration due to training[8-10], but they reported limited benefits. However, a recent study found that assisted bicycle training for the legs and arms delayed the secondary functional deterioration that can occur due to disuse among ambulatory and recently wheelchair-dependent boys.[13] We hypothesize that training may be less beneficial to older patients, who typically have less remaining muscle mass.[29] Therefore, patients should ideally begin training early in the course of the disease.

Because this was the first study of upper limb training with DAS in patients with DMD, we performed a feasibility study with a multiple N=1 design. Our study had a relatively small sample size (eight patients), and we did not compare our results with a control group. The external validity of our study is therefore limited, and the results should be interpreted with caution.[30] Larger, controlled trials are needed to expand these results and to further address the question of whether training with DAS can delay loss of arm motor function. These trials should include validated outcome measures of gross arm motor functions that are responsive to small changes over time. The primary outcome measure in our study (ARAT scores) was not completely compatible with the DAS device used, as the testing kit required for performing the tasks interfered with the device. This forced participants to use an unnatural movement pattern to perform the test when using the DAS. The MFM, a secondary outcome, was compatible with the DAS device, although this test has only a limited number of items for investigating shoulder movements.[21] No other suitable outcome measures of gross arm movements in DMD patients were available at the start of this study.[31] The recently developed Performance of the Upper Limb (PUL) can be useful to evaluate arm function in future clinical trials, since this tool assesses proximal as well as distal arm motor functions.[32] Its responsiveness to measures changes over time should however first be confirmed.

Implications for research

- Duchenne muscular dystrophy is a severe neuromuscular disorder leading to muscle weakness and loss of functional abilities
- Regular physical training is recommended to avoid secondary complications of physical inactivity, but training with resistance is often too hard for patients with severe muscle weakness
- This feasibility study showed that assisted upper limb training with dynamic arm support is a feasible and safe alternative to wheelchair-dependent patients
- Further research is required to determine whether an assisted upper limb training with dynamic arm support can delay the secondary functional deterioration

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Declaration of interest

We certify that MJ, MJ, NvA and IdG have no affiliations with or financial involvement with an organization or entity with a financial interest in, or financial conflict with, the subject matter or materials discussed in the manuscript. JB works for Focal Meditech B.V.. Focal Meditech B.V. financed the assembly of the dynamic arm supports and provided them on loan.

References

1. Emery AE. Population frequencies of inherited neuromuscular diseases--a world survey. Neuromuscul Disord 1991;1:19-29.
2. Petrof BJ. The molecular basis of activity-induced muscle injury in Duchenne muscular dystrophy. *Mol Cell Biochem* 1998;179:111-123.
3. Eagle M, Bourke J, Bullock R et al. Managing Duchenne muscular dystrophy--the additive effect of spinal surgery and home nocturnal ventilation in improving survival. *Neuromuscul Disord* 2007;17:470-475.
4. McDonald CM, Abresch RT, Carter GT et al. Profiles of neuromuscular diseases. Duchenne muscular dystrophy. *Am J Phys Med Rehabil* 1995;74:S70-S92.
5. Hiller LB, Wade CK. Upper extremity functional assessment scales in children with Duchenne muscular dystrophy: a comparison. *Arch Phys Med Rehabil* 1992;73:527-534
6. Mattar FL, Sobreira C. Hand weakness in Duchenne muscular dystrophy and its relation to physical disability. *Neuromuscul Disord* 2008;18:193-198.
7. Bushby K, Finkel R, Birnkrant DJ et al. Diagnosis and management of Duchenne muscular dystrophy, part 2: implementation of multidisciplinary care. *Lancet Neurol* 2010;9:177-189.
8. Scott OM, Hyde SA, Goddard C, Jones R, Dubowitz V. Effect of exercise in Duchenne muscular dystrophy. *Physiotherapy* 1981;67:174-176.
9. de Lateur BJ, Giaconi RM. Effect on maximal strength of submaximal exercise in Duchenne muscular dystrophy. *Am J Phys Med* 1979;58:26-36.
10. Vignos PJ, Jr., Watkins MP: The effect of exercise in muscular dystrophy. *JAMA* 1966; 197:843-848.
11. Bar-Or O, Rowland TW. Neuromuscular and musculoskeletal diseseases. In: Bar-Or O, Rowland TW, editors. *Pediatric exercise medicine. From physiologic principles to health care application.* United States of America: Human Kinetics; 2004. p 269-321.
12. Allen DG. Eccentric muscle damage: mechanisms of early reduction of force. *Acta Physiol Scand* 2001; 171: 311-319.
13. Jansen M, Alfen van N, Geurts ACH , Groot de IJM. Assisted Bicycle Training Delays Functional Deterioration in Boys With Duchenne Muscular Dystrophy: The Randomized Controlled Trial "No Use Is Disuse". *Neurorehabil Neural Repair* 2013;27:816-827.
14. Rahman T, Sample W, Seliktar R et al. Design and testing of a functional arm orthosis in patients with neuromuscular diseases. *IEEE Trans Neural Syst Rehabil Eng* 2007;15:244-251.
15. Romilly DP, Angelin C, Gosini RG, Hershler C, Raschka SU. A Functional Task Analysis and Motion Simulation for the Development of a Powered Upper-Limb Orthosis. *IEEE Transactions on Rehabilitation Engineering* 1994;2:119-129.
16. Jansen M, de Groot I, van Alfen N, Geurts AC. Physical training in boys with Duchenne Muscular Dystrophy: the protocol of the No Use is Disuse study. *BMC Pediatr* 2010;10:55.
17. van der Kooij, Prange GB, Krabbet T al. Preliminary results of training with gravity compensation of the arm in chronic stroke survivors. *Conf Proc IEEE Eng Med Biol Soc* 2009;2009:2426-2429.
18. Robertson RJ, Goss FL, Boer NF et al. Children's OMNI scale of perceived exertion: mixed gender and race validation. *Med Sci Sports Exerc* 2000;32:452-458.
19. Lyle RC. A performance test for assessment of upper limb function in physical rehabilitation treatment and research. *Int J Rehabil Res* 1981;4:483-492.
20. Yozbatiran N, Der-Yeghiaian L, Cramer SC. A standardized approach to performing the Action Research Arm Test. *Neurorehabil Neural Repair* 2008;22:78-90.
21. Berard C, Payan C, Fermanian J, Girardot F. A motor function measurement scale for neuromuscular diseases - description and validation study. *Rev Neurol* 2006;162:485-493.
22. Mathiowetz V., Weber G VG, Kashman N. Adult norms for the nine-hole peg test of finger dexterity. *Occup Ther J Res* 1985;5:24-38.
23. Jebsen RH, Taylor N, Trieschmann RB, Trotter MJ, Howard LA. An objective and standardized test of hand function. *Arch Phys Med Rehabil* 1969;50:311-319.
24. Brooke MH, Griggs RC, Mendell JR, Fenichel GM, Shumate JB, Pellegrino RJ. Clinical trial in Duchenne dystrophy. I. The design of the protocol. *Muscle Nerve* 1981;4:186-197.

25. Kumar A, Phillips MF. Use of powered mobile arm supports by people with neuromuscular conditions. *J Rehabil Res Dev* 2013;50:61-70.
26. Mastenbroek B, Haan de E,Berg van den M, Just L. *Herder Development of a Mobile Arm Support (Armon): Design Evolution and Preliminary Use Experience.* Proceedings of the 2007 IEEE 10th International Conference on Rehabilitation Robotics 2007. June 12-15, Noordwijk, the Netherlands.
27. Yasuda YK, Bowman K, Hsu JD. Mobile arm supports: criteria for successful use in muscle disease patients. *Arch Phys Med Rehabil* 1986;67:253-256.
28. Bartels B, Pangalila RF, Bergen MP, Cobben NA, Stam HJ, Roebroeck ME. Upper limb function in adults with Duchenne muscular dystrophy. J Rehabil Med 2011; 43:770-775.
29. Jansen M, van Alfen N, Nijhuis-van der Sanden MW, van Dijk JP, Pillen S, de Groot IJ. Quantitative muscle ultrasound is a promising longitudinal follow-up tool in Duchenne muscular dystrophy. *Neuromuscul Disord* 2012;22:306-317.
30. Bowen DJ, Kreuter M, Spring B. How we design feasibility studies. *Am J Prev Med* 2009;36: 452-457.
31. Mercuri E, McDonald C, Mayhew A et al. International workshop on assessment of upper limb function in Duchenne Muscular Dystrophy: Rome, 15-16 February 2012. *Neuromuscul Disord* 2012;22:1025-1028.
32. Mayhew A, Mazzone ES, Eagle M et al. Performance of the Upper Limb working group. Development of the Performance of the Upper Limb module for Duchenne muscular dystrophy. *Dev Med Child Neurol* 2013;55:1038-1045.

8

Summary and General discussion

Summary

Duchenne muscular dystrophy (DMD) is the most common muscular dystrophy in childhood and affects approximately 1 in 5000 newborn boys. Mutations in the *DMD* gene cause an absence of the functional protein dystrophin resulting in fragile muscle fibers and muscle wasting. Boys with DMD become wheelchair-dependent at the age of 9 to 10 years and soon thereafter they lose the ability to lift their arms against gravity. There is no cure for DMD yet and disease management is still symptomatic. An important aim in the rehabilitative management of DMD is to delay the loss of functional abilities such as walking. The loss of functional abilities is primarily the result of muscle wasting due to the disease, but it is also caused by 'disuse' secondary to a sedentary life style. For example, the increasing amount of energy needed to ambulate will (further) reduce the level of physical activity and, thus, cause secondary muscle wasting ('disuse atrophy'). International guidelines on physical training recommend boys with DMD to participate in regular 'gentle activities' to avoid disuse atrophy and other secondary complications of physical inactivity. However, these recommendations are vague and based on limited evidence. As for training, conventional resistance exercises will often be exhausting for the weak muscles of boys with DMD. So, in order to preserve optimal physical capacities, assisted physical training in which dynamic support is provided by a mechanic or electric device, such as an electric bicycle, could be a viable and safe solution for them. In the studies that constitute this thesis it was aimed to develop a feasible assisted physical training and to evaluate the effectiveness of this training in both ambulatory and wheelchair-dependent boys with DMD.

General introduction

Chapter 1 is the general introduction and provides background information on DMD. It gives a short overview of the pathophysiology, clinical progression, and treatment (including physiotherapy). It discusses the 'disuse' phenomenon, the possible role of physical training, and the need for new, validated clinical outcome measures that can monitor disease progression in both ambulant and wheelchair-dependent boys with DMD. Furthermore, it describes the aim and two main research questions of this thesis:

1) Are quantitative muscle ultrasound (QMUS) , the Assisted Six-Minute Cycling Test (A6MCT) and the KIDSCREEN-52 valid and/or responsive outcome measures to evaluate the effects of a physical training intervention in boys with DMD?
2) Is assisted physical training feasible, safe and effective to delay the functional deterioration in ambulatory and wheelchair-dependent children with DMD?

The general introduction ends with a description of the outline of this thesis. **Part 1** of the main body of this thesis describes the development and validation of the three *outcome measures* that were used to evaluate the effectiveness of assisted physical training, i.e. QMUS to assess muscle histology (chapter 2), the A6MCT to assess physical endurance

(chapter 3) and the KIDSCREEN-52 to assess Health Related Quality of Life (HRQoL) (chapter 4). In **part 2**, the protocol (chapter 5) and results (chapters 6 and 7) of two physical training programs are reported that together constitute the "*No Use is Disuse (NUD) study*" in boys with DMD. After part 2, the main findings of all studies are addressed in a general discussion, including their strengths and limitations. In addition, suggestions for future research are given.

Part 1 Outcome measures for Duchenne muscular dystrophy

The aim of **chapter 2** was to determine whether QMUS can be used as a follow-up tool in clinical trials for DMD. QMUS is a muscle imaging technique that reflects the dystrophic process by quantifying echo intensity (i.e. the mean grey level of ultrasound images) and muscle thickness. The echo intensity of boys with DMD shows a homogenous increase in echogenicity as a result of intramuscular fibrosis and fatty infiltration. It was hypothesized that quantitatively assessed echo intensity and muscle thickness are responsive enough to measure progressive changes in muscle structure over time and that these changes are associated with clinical disease progression and loss of function. In this study a longitudinal ultrasound/QMUS follow-up of the changes of two lower and two upper extremity muscles was performed in 18 boys with DMD. Additionally, the QMUS changes were compared with changes in clinical disease progression in 11 of these boys. Assessments of clinical disease progression included: the Vignos and Brooke functional rating scales, quantitative muscle strength testing, the Childhood Myositis Assessment Scale, and the Hammersmith Motor Ability Scale. The participating boys were aged 3.7 to 15.1 years; 15 of them were ambulatory and three where wheelchair-dependent. They each underwent a median of 4.5 QMUS measurements during a median follow up of 27.5 months. The results confirmed the hypothesis that muscle echo intensity significantly increased with clinical disease progression. The echo intensities of the rectus femoris and tibialis anterior muscles showed the highest responsiveness in our cohort, indicating that one has a good chance of finding a significant difference in echo intensity when repeating the measurement after one year. Somewhat counterintuitively, no significant decrease in muscle thickness was observed over time. Altogether, the results of this study establish QMUS for measuring echo intensity as a practical, child-friendly and valid tool for the longitudinal study and follow-up of boys with DMD.

Chapter 3 describes the development and validation of the A6MCT. The most frequently used submaximal endurance test for children with a neuromuscular disorder is the 6-Minute Walk Test (6MWT) that assesses the distance that a patient can quickly walk without running in 6 minutes. Endurance tests are the primary outcome measure in ongoing clinical trials in children with a neuromuscular disorder, because they correlate with functioning in daily life. The 6MWT is, however, not feasible for patients with Duchenne who are at the end of the ambulatory phase or who are wheelchair-depen-

dent. While controlled treatment trials are currently being developed for more severely affected children with DMD, no other suitable submaximal endurance test is yet available for this population, since standard bicycle ergometer use is too hard to sustain for affected muscles. In this perspective, the A6MCT was developed to assess submaximal endurance in children with severe muscle weakness. The A6MCT is a cycling test for the legs and arms using a motor-assisted mobility trainer with a no-load speed of 7 revolutions per minute (RPM). First, the development of the A6MCT is described. In the second part, the feasibility of the A6MCT is reported in healthy boys and in boys with DMD. For healthy boys, also the test-retest reliability was examined as well as the construct validity by showing the relationship between the A6MCT and the 6MWT. For boys with DMD, the relationship between the A6MCT and disease severity as assessed with the Motor Function Measure (MFM) was determined. The results showed that the A6MCT for the legs was feasible for 99% of the healthy boys and 97% of the boys with DMD. The A6MCT for the arms was feasible for all healthy boys and boys with DMD. Only two boys with DMD did not perform the A6MCT for the arms due to problems with their attention span. As expected, boys with DMD achieved fewer cycling revolutions than healthy boys. In healthy boys, the A6MCT was reproducible and positively correlated with the 6MWT for the legs ($r = 0.58$, $p < 0.01$) and arms ($r = 0.65$, $p < 0.01$). In the entire group of boys with DMD, the A6MCT for the legs positively correlated with the MFM ($\rho = 0.65$, $p < 0.01$). In the wheelchair-dependent subgroup, the A6MCT for the arms also positively correlated with the MFM ($\rho = 0.84$, $p < 0.01$). It was concluded that the A6MCT is the first ergometer test that can be performed by wheelchair-dependent children with severe muscle weakness due to a progressive neuromuscular disorder. It also seems to be a promising test for the longitudinal study and follow-up of children with neuromuscular disorders who are likely to lose their ability to walk.

Chapter 4 explores HRQoL in boys with DMD. HRQoL is an individual's perception of health and one's position in life. Authorities recommend the inclusion of a HRQoL assessment in clinical trials, because this increases insight in the perceptions of patients regarding their own health status. This chapter has a threefold aim. First, the composing factors of HRQoL were examined in 40 boys with DMD who were ambulant (n=19) or wheelchair-dependent with either good arm function (n=14) or decreased arm function (n=7). Then, the HRQoL perceptions of the boys were compared with the perception their parents had of them. Finally, the relationship between HRQoL, bodily functions and activities was determined. DMD was expected to negatively influence HRQoL, which then could be positively influenced by a new intervention. In this study, the KIDSCREEN-52 questionnaire was used to assess generic HRQoL of the boys and the proxy reports of their parents. The results of boys with DMD were compared with reference values of healthy age-matched Dutch boys. The results showed that boys with DMD perceived only their physical well-being to be lower compared to healthy controls. No clear correlations

between HRQoL, bodily functions and activities were found. This pattern of results indicates that, generally speaking, HRQoL is not negatively influenced by the existence of DMD or its progression. Parents, however, scored their sons much lower on the domains "Self perception", "Moods and emotions", and "Bullying" than the boys themselves. Because parents tend to underestimate the HRQoL of their sons with DMD, it seems important to counsel them about the preserved self-perceived HRQoL of these boys.

Part 2 Physical training in Duchenne muscular dystrophy

In **chapter 5**, the research questions, hypotheses en methods of the NUD study are presented. The NUD study is the first randomized controlled trial (RCT) in boys with DMD that examined whether low-intensity assisted physical training is beneficial in terms of preservation of muscle endurance and/or functional abilities. It was hypothesized that regular assisted physical activity would counteract the secondary functional deterioration as result of disuse. The NUD study consisted of two sub-studies that investigated the feasibility, safety and effectiveness of (1) dynamic leg and arm training for ambulant and recently wheelchair-dependent boys with DMD and (2) functional training with arm support for boys with DMD who had already been confined to a wheelchair for several years.

Study 1 (see chapter 6) was an RCT with multiple baseline measurements. It aimed to include 30 boys with DMD who were allocated to the intervention or control group. After a baseline period of eight weeks to control for initial disease severity and progression, the intervention group received assisted bicycle training for the legs and the arms, whereas the control group received usual care (no specific intervention) for 24 weeks. Boys in the intervention group trained 5 times per week, 15 minutes with their legs and 15 minutes with their arms using a mobility trainer with electric motor support . The training was performed at home or at school. The motor assistance was at least 7 RPM if no active motion was delivered. Boys were instructed to cycle at a continuous speed of approximately 65 RPM at an exertion ranging from "a little tired" to "getting more tired". Perceived exertion was assessed with the OMNI scale for perceived exertion. Boys in the control group received the same training after the control period. The intervention and control groups were followed up until 56 and 60 weeks, respectively, after study entry. The primary outcome measures were muscle endurance and functional abilities assessed with the A6MCT and MFM, respectively. Secondary outcome measures were the Pediatric Evaluation of Disability Inventory (PEDI), timed tests, muscle strength, range of joint motion, and QMUS. Adverse events during the intervention period were assessed by means of postal questionnaires and home visits paid by the researcher. Group differences between the intervention and control group were examined by an analysis of covariance with the primary endpoint being after 24 weeks of training/waiting list.

Study 2 (see chapter 7) was a non-controlled feasibility study that aimed to include ten wheelchair-dependent boys with DMD who had problems with reaching and lifting

their arms. After an eight-week period in which baseline information was obtained about the stability of the disease course, all boys were provided with a dynamic arm support for their non-dominant arm. Thereafter, they began a 24-week training program for their non-dominant arm. The program consisted of reaching exercises by using the dynamic arm support for playing a virtual reality computer game (5 days per week, 5 games per day) and for eating (at least twice a week). Efficacy was assessed after 12 weeks and at the primary endpoint after 24 weeks of training. A final follow-up assessment was performed 12 weeks after the end of the training. Compliance and safety were assessed via a questionnaire completed once every two weeks and home visits by the primary investigator. The primary efficacy outcome was the functional ability of the upper extremities assessed with the Action Research Arm Test (ARAT). Secondary outcome measures were the MFM dimension 3 "Distal motor function", the Nine-Hole Peg Test (9HPT), and the Jebsen Taylor Hand Function Test (Jebsen Test). The feasibility, safety and effectiveness of the DAS training program were described separately for each participant. The untrained dominant arm served as a within-subject reference.

The results of the NUD study were expected to increase insight into what type of physical training should be recommended to boys with DMD.

Chapter 6 describes the results of the RCT (see chapter 5) on dynamic leg and arm training. Thirty boys with DMD with a mean age of 10.5 years participated and were randomly allocated to either the intervention (n = 17) or the control group (n = 13). Eighteen boys were ambulant and 12 were wheelchair-dependent. Almost all the boys in the intervention group, except one wheelchair-dependent boy, completed the 24-week training program. After 24 weeks of training, the total MFM score had remained stable in the intervention group, whereas it had significantly decreased in the control group (group difference in time effect: Δ=4.9, 95%CI=2.2 to 7.6). No such training effects were found on the A6MCT for the legs (Δ = 5.6, 95% CI = −56.2 to 67.3), for the arms (Δ = 13.8, 95% CI = −60.7 to 88.3) or for the secondary outcome measures. After crossing over, eight of the 13 boys in the control group completed the same assisted bicycle training. After the training period, these eight boys did not show functional decline as assessed with the MFM either. No serious adverse events were reported in any of the participants. It was concluded that an assisted bicycle training of the legs and arms is feasible and safe for both ambulant and wheelchair-dependent boys with DMD and that prolonged physical training delays the secondary functional deterioration in these children.

In **chapter 7** the results of the non-controlled feasibility study (see chapter 5) are presented. Eight wheelchair-dependent boys, aged 12 to 20 years, received the dynamic arm support . Six of these eight boys completed the entire 24-week training program. The other two boys discontinued training due to practical reasons. Among the six boys who completed the program, four had a larger decrease in ARAT score in the untrained arm

(differences in ARAT score ranged from -4 to -21) compared to the trained arm (differences in ARAT score ranged from -13 to 6) after 24 weeks of training. This indicates that the trained arm retained more motor function than the untrained arm in four boys. Comparable results were found for the secondary outcomes. Only the Jebsen Test revealed no notable differences between the trained and untrained arms, with the exception of the item in which the boys had to pick up and drop small common objects. After 24 weeks of training, two participants lost the ability to pick up the objects with their untrained arm, although they retained the ability with their trained arm. No serious adverse events were reported during the study period. The results indicate that wheelchair-dependent boys with DMD with impaired arm function can still train their arms using dynamic arm support to preserve motor functions.

General discussion

This thesis started with two main research questions: (1) "Are Quantitative Muscle Ultrasound (QMUS), the Assisted Six-Minute Cycling Test (A6MCT) and the KIDSCREEN-52 valid and/or responsive outcome measures to evaluate the effectiveness of a physical training intervention in boys with Duchenne muscular dystrophy (DMD)?", and (2) "Is assisted physical training feasible, safe and effective to delay the functional deterioration in ambulatory and wheelchair-dependent children with DMD". Based on the results of the No Use is Disuse (NUD) study, it can be concluded that assisted physical training is feasible, safe and beneficial to delay the functional deterioration in boys with DMD across different stages of their disease. Although the QMUS, A6MCT and KIDSCREEN-52 are all outcome measures with good clinimetric properties, their use in the evaluation of the effectiveness of physical training in boys with DMD is still ambiguous and needs further investigation.

This thesis particularly focused on *assisted* training, as even low-resistance training is often too hard to sustain for children with DMD who suffer from severe muscle weakness. To this end, two assisted physical training programs were developed: (1) dynamic leg and arm training (assisted bicycle training) for ambulant and recently wheelchair-dependent boys with DMD, and (2) functional training with arm support for boys with DMD who have already been confined to a wheelchair for several years. Together, these two training programs constituted the NUD study. It was hypothesized that assisted physical training would counteract the secondary functional decline caused by disuse. Delaying the functional decline in boys with DMD is important, because these boys gradually lose the capacity to perform activities of daily living from a young age onwards. Only very recently, a promising drug under investigation in a phase III clinical trial (aimed at exon skipping of the genetic defect) failed to demonstrate a significant functional benefit, underscoring the persistent need for feasible and effective symptomatic treatments.

This general discussion first focuses on the potential underlying mechanisms of the observed beneficial effects of assisted physical training in the NUD study followed by suggestions to optimize the training. Thereafter, the pros and cons of the outcome measures selected to evaluate the effectiveness of assisted physical training will be discussed. This section is followed by issues concerning the clinical implementation of (assisted) physical training and related clinical assessments in the daily care of boys with DMD. Finally, some general conclusions will be drawn and recommendations for future research in this area will be provided.

The disuse hypothesis: what do we know?

The results of the NUD study (chapters 6 and 7) showed that assisted physical training is feasible and safe for ambulant and wheelchair-dependent boys with DMD. This is in

accordance with previous studies on sub-maximal resistance training in boys with DMD[1-3] and some of the studies on voluntary exercise training in mdx mice[4-6], the most commonly used mouse model in DMD research. These previous studies concluded that sub-maximal resistance training and voluntary exercises did not cause any harm, but the studies in boys with DMD did not show a clear functional benefit either. The NUD study reported in chapter 6 is, therefore, the first training study to show a significant functional effect of low resistance (assisted bicycle) training in boys with DMD: it prevented a 6.3% functional deterioration of the total Motor Function Measure (MFM) score during 24 weeks compared to a control group receiving no such training.

Although relatively small, this preventive effect occurred in a period of merely a half year, which warrants the speculative question what such exercise training would do when maintained over a lifespan compared to an inactive lifestyle. Unfortunately, investigating a life-long intervention is virtually impossible using a randomized controlled design. As a consequence, 'formal' evidence (based on RCTs) for the benefit of life-long, low-to-moderate resistance training in boys with DMD will not readily become available. Recently, our group was able to show that an active lifestyle induced by either 16-weeks aerobic exercise training or individually tailored cognitive behavior therapy showed long-lasting (28 weeks) beneficial effects on fatigue and physical activity compared to no intervention in patients with facioscapulohumeral dystrophy (Facts-2-FSHD study).[7] This result was obtained even though the physical exercise did not reach the level recommended by the American College of Sports Medicine[8] in many participants of both intervention groups (Voet et al., submitted). Remarkably, the observed functional improvements were coincided by less fatty degeneration of thigh muscles assessed by magnetic resonance imaging (MRI) in both intervention groups compared to the control condition (Janssen et al., in preparation). Together, the NUD study and the Facts-2-FSHD study seem to point towards two interesting notions: (1) beneficial effects at both a functional and a muscular level can be achieved by physical training of low-to-moderate intensity in persons with muscular dystrophy of various age groups, and (2) the training effects may be relatively small over a period of approximately a half year, but might amount to a much larger size when observed over a substantial period of the lifespan. Since the literature is consistent about the safety of low-to-moderate physical exercise in patients with muscular dystrophy[9], we propose to advise these patients a lifestyle as actively as possible, without overexertion (see further: recommendations). Future studies should try to compare the natural course of muscular dystrophies and their functional consequences between 'active' and 'inactive' patients to provide definitive evidence for the legitimacy of this advice.

The important question remains, however, *how* the preservation of functional abilities through assisted bicycle training as observed in the NUD study can be explained. Since it is unlikely that the training positively influenced the lack of dystrophin, it is plausible that the training counteracted disuse at several physiological levels. In this thesis,

disuse has been defined as "a discrepancy between individual (latent) capacity and actual performance in daily life, as a result of a sedentary life style". Such disuse is commonly present in boys with DMD contributing to a loss of functional abilities beyond the degree that might be predicted based on the muscle pathology itself.[10] The effects of disuse due to a lack physical activity may, as a matter of 'physiological law', occur in (1) the neuromuscular system, (2) the cardiovascular system, (3) the joint-ligamental-skeletal system, and/or (4) the central nervous system.

Neuromuscular system

At the neuromuscular level, it is possible that the assisted bicycle training prevented part of the muscle atrophy secondary to disuse. Muscle atrophy is defined as a decrease in muscle mass leading to a loss of muscle strength and increased muscular fatigability.[11-13] Indeed, there are indications that a combination of static and dynamic resistance exercises can reduce muscle atrophy during unloading.[14] However, the assisted bicycle training did not significantly affect muscle strength or quantitative muscle ultrasound (QMUS) in the participants of the NUD study. This lack of observed effect at the neuromuscular level might be explained by the fact that the training was not primarily aimed at muscle strengthening, but rather at reducing physical inactivity. It is still possible that the assisted bicycle training increased the number of oxidative muscle fibers and reduced muscle fatigability[15], since these neuromuscular characteristics were not assessed in our study.

Although yet to be proven, several neuromuscular mechanisms of DMD pathology may have been beneficially influenced by the increased level of physical activity as hypothesized by Markert et al. (2011)[16], including (1) mechanical weakening of the sarcolemma, (2) inappropriate calcium influx, (3) aberrant cell signalling, (4) increased oxidative stress, and (5) recurrent muscle ischemia. For example, chronic exercises may temporarily enhance muscle regeneration and repair in the early stages of the disease.[17] Another example is that low-intensity exercises may reduce markers of oxidative stress by increased antioxidant activity.[18] Nevertheless, cellular influences of exercise in DMD remain speculative and sensitive biomarkers are needed to underscore such underlying mechanisms.[16]

Cardiovascular system

Another possibility is that the assisted bicycle training prevented or reversed some of the disuse effects within the cardiovascular system. This possibility is, however, questionable. The cardiovascular response to disuse includes a decreased maximum oxygen uptake, which is defined as "the highest volume of oxygen that can be consumed by the body per time unit".[19,20] In healthy children, a physical training at ~80% of the maximum heart rate can improve the maximum oxygen uptake by increasing the cardiac output.[20,21] In boys with DMD, however, the main limiting factor of a decreased maximum oxygen uptake is the reduced muscle mass rather than the respiratory or cardiac function.[22] This notion

implies that boys with DMD often reach a very low peak heart rate during conventional ergometry[22] and potentially also during training. Therefore, the intensity of the assisted bicycle training that the participants of the NUD study perceived as "easy" to "somewhat easy" may have been too low to impose serious demands on the aerobic capacity, which would explain the absence of a significant training effect on the Assisted Six-Minute Cycling Test (A6MCT), a submaximal endurance test reported in chapter 3. On the other hand, the low-intensity assisted bicycle training may have had peripheral effects on the vascular system, for instance a reduction in vascular resistance in the extremities, as occurs in healthy human beings.[23] Such effects were not assessed in the NUD study and may be limited in boys with DMD, since the lack of dystrophin causes a loss of neuronal nitric oxide synthase (NOS) which may result in muscle ischemia, and no reactive vasodilatation, when the muscles are exercised.[24,25]

Joint-ligamental-skeletal system

Although in the NUD study no significant effect of assisted bicycle training was found on the well-known flexion contractures of the hip, knee and elbow, and plantarflexion contracture of the ankle[26], the training could be a positive addition to the current set of stretching techniques. A combination of stretching techniques, including active stretching, active-assisted stretching, passive stretching, and prolonged elongation using orthoses and/or standing devices may delay the development of contractures in the early stages of DMD.[27,28] Assisted cycling would be a form of active-assisted stretching without the need of help from a parent or a physiotherapist. Stretching is usually recommended to be performed four to six days per week, which was the case for assisted bicycle training in our study.

Central nervous system

Also at the level of the central nervous system, beneficial changes may have occurred as a result of increased physical activity, such as improved coordination of extremity muscles, as a result of which performance on the MFM may have been improved. Indeed, it is well known that functional exercises not only induce peripheral adaptations of the neuromusculoskeletal and cardiovascular systems, but also central (e.g. cortical) adaptations of sensorimotor representations and motor programs underlying improved motor performance.[29-31] Tests to assess such underlying mechanisms were, however, not included in the NUD study.

Combined effects

A last possibility is that, even though the assisted bicycle training did not lead to measurable training effects at the muscular, cardiovascular or joint-ligamental-skeletal levels, several insignificant trends towards improvement in all systems together may have constituted a significant effect at the performance level of the MFM as a result of assisted

bicycle training. Whether this calls for the development of more sensitive outcome measures directed at each individual system or whether this should be regarded as a plea for functional (composite) outcome measures requiring the use of multiple systems can be debated. In the case of clinical benefit, researchers will most likely emphasize the relevance of the identification of underlying determinants, whereas clinicians may be satisfied with improved functional outcome in its own right.

The working mechanism by which assisted physical training delays the functional deterioration in boys with DMD is still unclear. Insight in underlying mechanisms may help to develop and improve (new) training interventions.

Optimizing physical training: 'one size fits all' or a personalized approach?

Although the training intensity of both the assisted bicycle training (chapters 6 and 8) and the functional arm training with dynamic arm support (chapter 7) were individually determined, the frequency and type of training were the same for each participant. This standardized approach is thought to be essential to evaluate the effectiveness of an intervention in a clinical trial, but it raises the question whether a more personalized approach should be used when implementing training programs in daily care, since the response to a certain physical training may differ per individual. A personalized approach means that the intervention (training frequency, intensity and type) is tailored to the individual capacities, which is the opposite of the 'one-size-fits-all' principle.[32,33]

Frequency of training

The boys (chapters 6 and 7) who participated in the assisted physical training programs were instructed to train five times per week. This high training frequency was chosen to achieve as much increase in physical activity as possible to prevent or reverse disuse. Indeed, the World Health Organization (WHO) recommends sixty minutes of moderate-to-vigorous daily physical activity to maintain well-loadable musculoskeletal tissues in healthy children.[34] Taken into account that patients with a neuromuscular disorder are often sedentary, suffering from poor physical fitness, it is questionable whether training less than five days per week could be meaningful. It is, nevertheless, possible that there are boys with DMD who have already adopted a physically active lifestyle, which would reduce the need to participate in a training program for five times a week. Training less frequently (e.g. three times a week) might be enough for them. Still, the assisted bicycle training was beneficial for 24 of the 30 participants for whom the training was additional to their habitual sports activities such as swimming (n=15) and wheelchair-hockey (n=8) in which they participated once or twice a week.

Intensity of training

The training intensity of the assisted physical training programs was low-to-moderate as determined with the OMNI scale for perceived exertion.[35] A low-to-moderate training intensity was chosen, because of the positive effects of voluntary low-intensity physical training on fatigue resistance in mdx mice. Although a low-to-moderate intensity may be sufficient for children who are in different stages of DMD, a higher training load may be needed for the young ambulant boys than for the older wheelchair-dependent boys. Yet, caution is warranted to prevent exhausting high-resistance exercises that may increase the mechanical stress on the muscle fibers and may, therefore, negatively influence the progression of DMD.[36-38] Although in the NUD study the OMNI scale for perceived exertion was used to determine the training intensity, it would be worthwhile to search for more objective tools. Hearth rate was not used to establish the training intensity in the assisted bicycle training, because it was considered to be impossible to validly determine the maximum heart rate in boys with DMD due to reasons explained above (see cardiovascular system). The results of chapter 3 showed, however, that boys with DMD can achieve comparable maximum heart rates as healthy boys during sub-maximal endurance tests, but these results were achieved during the study and the study protocol has not been changed during the study.

Type of training

Although two types of assisted physical training were investigated in the NUD study, other types of (assisted) physical training such as cycling outdoors on an electric bicycle should be considered. It is important that these activities are joyful, besides effective. This point was underscored by all eight boys who played the virtual reality game as part of their arm training (chapter 7). They did not like the game anymore after 24 weeks of training which resulted in non-adherence. Conversely, a physical training that is meaningful and pleasurable will enhance compliance. This is important since boys with DMD are to be recommended to implement physical training into their daily lives from a young age onwards. The high level of adherence in the assisted bicycle training study confirms that good compliance is possible. It is also indicative of the notion that boys with DMD are willing to play an active role in their disease management. The assisted-physical-training facilities for wheelchair-dependent boys should be extended, especially with a growing population of older patients with DMD having upper extremity impairments. One could think of three dimensional arm training while using a dynamic arm support. For example, boys can play standard virtual-reality computer games or sports activities such as table tennis while using a mechanical support that assists the arm through the entire range of motion. Also power-assisted manual wheelchairs might be considered as training devices.

Further research is needed to develop specific guidelines including frequency, intensity, and type of physical training in boys with DMD.

Clinical outcome measures: poor results or poor responsiveness?

The selection of outcome measures for studies in patients with DMD receives a lot of attention in literature[39-42], in particular with regard to several ongoing and planned efficacy studies on pharmacological treatments. Outcome measures should be responsive to measure small but relevant changes in disease progression that are related to clinical milestones such as the loss of walking ability. Moreover, outcome measures should be appropriate for various stages of DMD. From this point of view, composite scales of motor functioning reflecting the capacity and performance of several activities of daily living are thought to be useful. This is why the primary outcome measures in both sub-studies of the NUD study were composite scales of motor functioning: the MFM[43] and the Action Research Arm Test (ARAT).[44-45]

The MFM was the only outcome measure that showed a significant effect of assisted bicycle training in the NUD study. The MFM is a clinical test that assesses (1) standing positions and transfers as well as (2) axial and proximal motor functions and (3) distal motor functions, of the arms and legs in ambulant and wheelchair-dependent patients with a neuromuscular disorder.[43] Although the MFM was sensitive enough to measure training-induced group differences, a disadvantage of the MFM is that it is a generic test.[39] The MFM has not been specifically designed for patients with DMD, which is reflected in, for example, the limited number of items on upper-arm functioning. Only three items measure activities needing antigravity strength in the shoulder: touching a bal, moving the hands to the head, and reaching for a pencil. This can be considered a limitation with regard to a population in which proximal arm functions are more affected than distal arm functions.[46] It might be that composite scales that are more specifically designed for DMD, such as the Performance of the Upper Limb (PUL)[47] and the North Start Ambulatory Assessment (NSAA)[48], would have been (even) more responsive to measure changes in motor functioning over time. However, the participants in the NUD study were both ambulant and wheelchair-dependent and they trained their legs as well as their arms. Thus, neither the PUL nor the NSAA would have been an appropriate primary outcome. The current advantage of the MFM is its applicability to assess all main domains of motor functioning in both ambulant and wheelchair-dependent boys.

None of the secondary outcome measures showed a significant difference between the intervention and control group after 24 weeks of assisted bicycle training. As described above, this could be explained either by an absence of training effects on the secondary

outcomes or by a limitation of the outcome measures themselves. For instance, echo intensity as assessed with QMUS showed a good sensitivity to time effects during a 1-year follow-up (chapter 2), but did not show a significant training-related group difference (chapter 6). This could reflect that there was indeed no training effect on the quality of the muscle tissue, but it is also possible that the follow-up of 24 weeks was too short to measure significant change. In addition, assessing more 'DMD-specific' muscles, such as the deltoid muscle, might have increased sensitivity of QMUS. It is also possible that MRI would have been a more sensitive alternative since MRI can measure progression of fat replacements already after nine months in boys with DMD.[49,50] MRI has, however, the disadvantages of being much more expensive and requiring sedation in young children. In any case, for future studies, muscle imaging is expected to be a relevant supplement to the clinical tests in order to objectify structural muscle changes in boys with DMD, without influence of cooperation or fatigue.

The training-related preservations of motor functions in the NUD study were also not paralleled by significant preservations in physical endurance as assessed with the A6MCT. This can probably be explained by the relatively low training intensity of the assisted bicycle training, limiting the impact on the aerobic capacity. It could also be related to a limited reproducibility of the A6MCT in boys with DMD. Although the A6MCT showed good reproducibility in healthy boys (chapter 3), it might be that its reproducibility in boys with DMD is lower, because they are less used to bicycle than healthy boys. This should be further studied. Furthermore, the performance on the A6MCT is influenced by motivation and cooperation, which can be tough for young boys. Comparable results have been found in clinical trials using the Six-Minute Walk Test (6MWT) as a primary outcome. Although the 6MWT has shown good psychometric properties[51,52], it has a large standard deviation and just very recently a clinical trial on exon-skipping in boys with DMD failed to demonstrate that the drug drisapersen could significantly improve the distance walked.

The need to assess Health Related Quality of Life (HRQoL), an individual's perception of his own health, in clinical trials is widely recognized.[53,54] The NUD study included the KIDSCREEN-52 that showed that the HRQoL of boys with DMD was similar to age- and gender-matched healthy controls (chapter 4). In accordance with previous studies, only the dimension "physical well being" was lower in boys with DMD.[55-57] The results of chapter 4 are also in line with the finding that HRQoL remains constant over time and, thus, is not related to disease severity.[55] Although these findings are positive and indicate that boys with DMD keep being satisfied with their lives, they are also counterintuitive in a population that gradually loses the ability to participate in several physical and social activities.[54] It may, therefore, be that the questionnaires used to assess HRQoL in boys with DMD, including the KIDSCREEN-52 and the Pediatric Quality of Life Inventory (PedsQL), are not sensitive enough to measure changes over time. An explanation for the possibly limited

sensitivity of the KIDSCREEN-52 and PedsQL may be that these questionnaires assess to what extend a child has had a problem had with a certain activity during the past month rather than asking for a more absolute measure of difficulty of the activity.[52] A recently published study showed that the Pediatric Society of North America (POSNA) Pediatric Outcomes Data Collection Instrument (PODCI), a self-report or proxy-measure of functional abilities, may be a better questionnaire for use in clinical trials on DMD, since it is able to detect one-year changes that are more related to the 6MWT than the PedsQL.[52]

The ideal set of outcome measures to evaluate the effectiveness of physical training or drug treatment in boys with DMD is still a matter of debate, since the quality of many clinical outcomes is uncertain. There is an urgent need for a core set of valid, reliable and responsive outcome measures in clinical DMD research.

Implementation of physical training and clinical assessment in daily practice: what can be recommended?

There has been a long-lasting debate about the recommendation of physical training to boys with DMD because of the putative fragility of the muscle fibers. This is the reason that many boys with DMD, as well as their families and therapists, may be worried that physical training might accelerate disease progression. There is, however, besides the NUD study (chapter 6), increasing evidence that low-to-moderate physical training in persons with neuromuscular disease is safe and beneficial to preserve motor functions, reduce fatigue and improve physical activity (Voet et al., submitted).[9] The implementation of (assisted) physical training, as part of an active life-style, should therefore be promoted in boys with DMD to prevent disuse and other complication of deconditioning.[28] Physiotherapists and rehabilitation physicians should inform the boys and parents about the do's and don'ts of physical training. Although not proven in clinical studies in humans, based on the pathophysiology of DMD, the results of training studies in mdx mice, and on clinical experience, it can be recommended to refrain from (eccentric) exercises with a high mechanical load.[28,58-62] Furthermore, physiotherapists should help the boys to find an appropriate type and intensity of (assisted) physical training that is also pleasant and motivating. It is the responsibility of the boys and parents to actually become and sustain being physically active, taking into account the individual physical limitations.

Specific (inter)national guidelines for physical therapy and physical training in DMD do not exist[63], which makes it hard to know what physiotherapists already do in their taking care of boys with DMD. Do they already recommend them to become physically active? More particularly, is (assisted) physical training part of their treatment advice? The implementation of (assisted) physical training in the daily routine of boys with DMD may actually reduce the need for physiotherapy. In the NUD study, the participants visited a physiotherapist

once or twice a week. Yet, frequent, life-long, visits to the physiotherapist are burdensome and expensive or may result in 'therapy fatigue' and non-compliance. The implementation of routine (assisted) physical training may, therefore, enhance the responsibility of the boys and their parents for disease management and (partly) shift the role of the physiotherapist from 'traditional therapist' towards 'coach' and 'assessor'.

The role of the physiotherapist as a coach and assessor is important to monitor disease progression and allow for well-timed interventions and application of facilities and medical devices by appropriate referral to an occupational therapist and/or rehabilitation physician. Furthermore, the physiotherapist can evaluate the effects of interventions over a prolonged period. Table 1 gives an overview of the outcome measures that can be used in a regular community-based physiotherapy practice to monitor boys with DMD at different disease stages. For additional assessments, such as muscle imaging and spirometry, patients should be referred to specialized DMD centres. If possible, routine clinical evaluations should take place every 6 months. Because these evaluations will take on average two hours (including breaks), they could be spread out over two appointments. An alternative is to conduct a complete assessment once a year and a selection of the assessments every six months. Such a selection might consist of a motor function scale and timed functional tests, which will reduce the administration time to approximately 30-40 minutes. Because not all the recommended tests and questionnaires are freely available on the internet, their implementation in daily practice may be hampered.

Regular (assisted) physical training with a low-to-moderate intensity should be recommended to boys with DMD in order to counteract the negative effects of disuse. It seems advisable to avoid (eccentric) exercises with a high mechanical load.

Table 1 Recommended outcome measures for the different stages in the course of DMD

Domain	Outcome measures for ambulant boys	Outcome measures for wheelchair-dependent boys
Muscle strength	Quantitative muscle testing of the lower (quadriceps and hamstring muscles and ankle dorsiflexors) and upper extremity (biceps, triceps and deltoid muscle), or use manual muscle testing if the muscle is not able to move against gravity (MRC <3)	Quantitative muscle testing of the upper extremity (biceps, triceps and deltoid muscle, and grip strength), or use manual muscle testing if the muscle is not able to move against gravity (MRC <3)
Range of motion	Goniometry of the lower (hip extension, knee extension and ankle dorsiflexion) and upper extremity (elbow extension)	Goniometry of the upper extremity (elbow extension, supination and pronation, wrist extension and finger extension)
Endurance	6MWT	A6MCT
Motor function	Vignos lower extremity scale and Brooke upper extremity scale Composite motor function scales for the lower, and preferably also the upper, extremity (MFM, or NSAA and PUL) Timed functional tests including the time to rise from the floor, the time to walk 10 meter, and the time to climb 4 standard stairs	Brooke upper extremity scale, and Vignos lower extremity scale if the boy is still able to stand for a transfer Composite motor function scales for the upper extremity (PUL), and also the trunk motor functions in recently wheelchair-dependent boys (MFM)
Activities of daily living	Mobility, self-care and social functioning (PEDI, or potentially the recently developed PROM)	Self-care and social functioning (PEDI, ABILHAND, and/or potentially the recently developed PROM)
Participation	CAPE from 6 years onwards	CAPE
Health Related Quality of Life	PedsQL Neuromuscular module, or PODCI	PedsQL Neuromuscular module, or PODCI

MRC, Medical Research Council; 6MWT, Six-Minute Walk test; A6MCT, Assisted Six-Minute Cycling Test; MFM, Motor Function Measure; NSAA, North Star Ambulatory Assessment; PUL, Performance of the Upper Limb; PEDI, Pediatric Evaluation of Disability Inventory; PROM, Patient Reported Outcome Measure; CAPE, Children's Assessment of Participation and Enjoyment; PODCI, Pediatric Outcomes Data Collection Instrument
(Table based on Bushby et al. (2010), and adjusted.)

Conclusions and future research

The NUD study comprises the first randomized controlled trial (RCT) on assisted physical training in boys with DMD. The results have filled part of the current gap of knowledge about the safety and effectiveness of physical training in DMD. In addition, it has increased insight into the applicability of several clinical measures to evaluate the effectiveness of treatments.

Further research is required to gain insight in the working mechanisms underlying the training-induced preservation of functional abilities. Further research is also recommended to increase insight in which type of training should be recommended to boys with DMD: what exactly should they do at which stage of the disease? For this purpose, an upper limb training program with dynamic arm support is currently being developed for boys with DMD who experience difficulties in lifting their arms. In this new RCT, the clinical evaluator will be blinded to treatment allocation. Furthermore, strict inclusion criteria will be applied and stratified group randomization (able to lift the arms above the head vs. unable to lift the arms above head but able to bring the hands to the mouth) will be used to reduce the phenotypic variability in each group.

Future clinical trials on physical training should use responsive outcome measures that are meaningful to the boys and their parents. With an increasing population of older wheelchair-dependent boys with DMD, there is a need to develop and validate a tool that can be used across the different disease stages and assess the specific abilities affected in boys with DMD. Recently, the Performance of the Upper Limb (PUL) module for DMD has been developed[47], which covers the whole spectrum of upper limb disabilities in these boys. Further research is needed on the responsiveness of the PUL. In addition, it deserves further investigation whether the echo intensity of 'DMD-specific' muscles, including the deltoid muscle, is more responsive to disease progression and effects of interventions. The A6MCT, a test of physical endurance, should be further standardized and its validity in older boys with DMD should be established. Next to the A6MCT, accelerometers can be used to gain insight into the level of daily physical activity in DMD.[64] Above all, outcome measures should be selected carefully and be restricted to relevant clinical characteristics, since they are often time consuming and confronting for the boys as well as their parents.

Future clinical trials should ideally use stratified randomization to allocate participants to either the intervention or control group according to DMD phenotype: the 'common', 'mild' or 'severe' form.[65] This is, however, difficult with a relatively low number of approximately 500 patients in the Netherlands. Other study designs than RCTs, such as a comparison of an experimental group to a large and representative natural cohort, should therefore be considered. Also large prospective cohort studies on different treatment

regiments could be valuable, especially in a time that multiple clinical trials are being conducted and patients and their families may be overwhelmed with research.

Lastly, knowledge about the relationship between the level of dystrophin and functional abilities is needed by conducting studies that aim to (partially) restore the level of dystrophin in the muscles. A recent study among adults with Becker muscular dystrophy showed that the level of dystrophin is an important determinant of disease severity, however only if the level of dystrophin is below 10%.[66] There is still a lack of knowledge of the relationship between the level of dystrophin and the ability to exercise. A case-study of a girl with DMD (see Appendix) gives some insight into this matter by showing that assisted physical training is feasible and probably beneficial also for a girl who expresses a low level of dystrophin. It seems also relevant to combine drug treatment with physical training in order to investigate whether (partial) restoration of dystrophin levels can optimize training effects.

References

1. Vignos PJ, Jr., Watkins MP. The effect of exercise in muscular dystrophy. *JAMA* 1966; 197:843-848.
2. de Lateur BJ, Giaconi RM. Effect on maximal strength of submaximal exercise in Duchenne muscular dystrophy. *Am J Phys Med* 1979;58:26-36.
3. Scott OM, Hyde SA, Goddard C, Jones R, Dubowitz V. Effect of exercise in Duchenne muscular dystrophy. Physiotherapy 1981;67:174-176.
4. Call JA, McKeehen JN, Novotny SA, Lowe DA. Progressive resistance voluntary wheel running in the mdx mouse. *Muscle Nerve* 2010;42:871-880.
5. Hayes A, Williams DA. Beneficial effects of voluntary wheel running on the properties of dystrophic mouse muscle. *J Appl Physiol* 1996;80:670-679.
6. Dupont-Versteegden EE, McCarter RJ, Katz MS. Voluntary exercise decreases progression of muscular dystrophy in diaphragm of mdx mice. *J Appl Physiol* (1985) 1994;77:1736-1741.
7. Voet NB, Bleijenberg G, Padberg GW, van Engelen BG, Geurts AC. Effect of aerobic exercise training and cognitive behavioural therapy on reduction of chronic fatigue in patients with facioscapulohumeral dystrophy: protocol of the FACTS-2-FSHD trial. *BMC Neurol* 2010;10:56.
8. Haskell WL, Lee IM, Pate RR et al. Physical activity and public health: updated recommendation for adults from the American College of Sports Medicine and the American Heart Association. *Med Sci Sports Exerc* 2007;39:1423-1434.
9. Voet NB, van der Kooi EL, Riphagen II, Lindeman E, van Engelen BG, Geurts AC. Strength training and aerobic exercise training for muscle disease. *Cochrane Database Syst Rev* 2013;7:CD003907.
10. McDonald CM. Physical activity, health impairments, and disability in neuromuscular disease. *Am J Phys Med Rehabil* 2002;81:S108-S120.
11. Edgerton VR, Roy RR, Allen DL, Monti RJ. Adaptations in skeletal muscle disuse or decreased-use atrophy. *Am J Phys Med Rehabil*;2002:81:S127-147.
12. Jackman RW, Kandarian SC. The molecular basis of skeletal muscle atrophy. *Am J Physiol Cell Physiol* 2004;287:C834-843.
13. Bodine SC. Disuse-induced muscle wasting. *Int J Biochem Cell Biol* 2013;45:2200-2208.
14. Adams GR, Haddad F, Bodell PW, Tran PD, Baldwin KM. Combined isometric, concentric, and eccentric resistance exercise prevents unloading-induced muscle atrophy in rats. *J Appl Physiol (1985)* 2007;103:1644-1654.
15. Hayes A, Lynch GS, Williams DA. The effects of endurance exercise on dystrophic mdx mice. I. Contractile and histochemical properties of intact muscles. *Proc Biol Sci* 1993;253:19-25.
16. Markert CD, Ambrosio F, Call JA, Grange RW. Exercise and Duchenne muscular dystrophy: toward evidence-based exercise prescription. *Muscle Nerve* 2011;43:464-478.
17. Okano T, Yoshida K, Nakamura A et al. Chronic exercise accelerates the degeneration-regeneration cycle and downregulates insulin-like growth factor-1 in muscle of mdx mice. *Muscle Nerve* 2005;32:191-199.
18. Kaczor JJ, Hall JE, Payne E, Tarnopolsky MA. Low intensity training decreases markers of oxidative stress in skeletal muscle of mdx mice. *Free Radic Biol Med* 2007;43:145-154.
19. Bortz WM, 2nd. The disuse syndrome. *West J Med* 1984;141:691-694.
20. Bar-Or O, Rowland TW. Physiologic and perceptual responses to exercise in healthy child. In: Bar-Or O, Rowland TW, editors. *Pediatric exercise medicine. From physiologic principles to health care application.* United States of America: Human Kinetics; 2004. p 3-59.
21. Rotstein A, Dotan R,Bar-Or O, Tenenbaum G. Effect of training on anaerobic threshold, maximal aerobic power and anaerobic performance of preadolescent boys. *Int J Sports Med* 1986;7: 281-286.
22. Bar-Or O, Rowland TW. Neuromuscular and musculoskeletal diseseases. In: Bar-Or O, Rowland TW, editors. *Pediatric exercise medicine. From physiologic principles to health care application.* United States of America: Human Kinetics; 2004. p 269-321.
23. Thijssen DH, Maiorana AJ, O'Driscoll G, Cable NT, Hopman MT, Green DJ. Impact of inactivity and exercise on the vasculature in humans. *Eur J Appl Physiol* 2010;108:845-875.
24. Thomas GD, Ye J, de Nardi C, Monopoli A, Ongini E, Victor RG. Treatment with a nitric oxide-donating NSAID alleviates functional muscle ischemia in the mouse model of Duchenne muscular dystrophy. *PLoS One* 2012;7:e49350.

25. Brenman JE, Chao DS, Xia H, Aldape K, Bredt DS. Nitric oxide synthase complexed with dystrophin and absent from skeletal muscle sarcolemma in Duchenne muscular dystrophy. *Cell* 1995; 82:743-752.
26. McDonald CM, Abresch RT, Carter GT et al. Profiles of neuromuscular diseases. Duchenne muscular dystrophy. *Am J Phys Med Rehabil* 1995;74:S70-S92.
27. Scott OM, Hyde SA, Goddard C, Dubowitz V. Prevention of deformity in Duchenne muscular dystrophy. A prospective study of passive stretching and splintage. *Physiotherapy* 1981;67:177-180.
28. Bushby K, Finkel R, Birnkrant DJ et al. Diagnosis and management of Duchenne muscular dystrophy, part 2: implementation of multidisciplinary care. *Lancet Neurol* 2010;9:177-189.
29. Mulder T. Motor imagery and action observation: cognitive tools for rehabilitation. *J Neural Transm* 2007;114:1265-1278.
30. Schmidt RA. Motor schema theory after 27 years: reflections and implications for a new theory. *Res Q Exerc Sport* 2003;74:366-375.
31. Shumway-Cook A, Woollacott MH. Physiological basis of motor learning and recovery in function. In: Shumway-Cook A, Woollacott MH, editors. *Motor control. Translating research into clinical practice.* 3th ed. United States of America: Lippincott Williams and Wilkins; 2007. p 83-99.
32. Edouard P, Gautheron V, D'Anjou MC, Pupier L, Devillard X. Training programs for children: literature review. *Ann Readapt Med Phys* 2007;50:510-9, 499-509.
33. Takken T. Fysieke activiteit bij kinderen. In: Takken T. van Brussel M, Hulzebos HJ, editors. *Inspanningsfysiologie bij kinderen*. Houten: Bohn Stafleu van Loghum; 2008. p 42-48.
34. World Health Organization. *Global Strategy on Diet, Physical Activity and Health. Physical Activity and Young People.* Geneva: WHO 2010. Available at http://whqlibdoc.who.int/publications/2010/9789241599979_eng.pdf. Accessed November 6th, 2013.
35. Robertson RJ, Goss FL, Boer NF et al. Children's OMNI scale of perceived exertion: mixed gender and race validation. *Med Sci Sports Exerc* 2000;32:452-458.
36. Bushby K, Finkel R, Birnkrant DJ et al. Diagnosis and management of Duchenne muscular dystrophy, part 2: implementation of multidisciplinary care. *Lancet Neurol* 2010;9:177-189.
37. Ansved T. Muscle training in muscular dystrophies. *Acta Physiol Scand* 2001;171:359-366.
38. Ansved T. Muscular dystrophies: influence of physical conditioning on the disease evolution. *Curr Opin Clin Nutr Metab Care* 2003;6:435-439.
39. Mercuri E, Mayhew A, Muntoni F et al. Towards harmonisation of outcome measures for DMD and SMA within TREAT-NMD; report of three expert workshops: TREAT-NMD/ENMC workshop on outcome measures, 12th--13th May 2007, Naarden, The Netherlands; TREAT-NMD workshop on outcome measures in experimental trials for DMD, 30th June--1st July 2007, Naarden, The Netherlands; conjoint Institute of Myology TREAT-NMD meeting on physical activity monitoring in neuromuscular disorders, 11th July 2007, Paris, France. *Neuromuscul Disord* 2008;18:894-903.
40. Bushby K, Connor E. Clinical outcome measures for trials in Duchenne muscular dystrophy: report from International Working Group meetings. Clin Investig (Lond) 2011;1:1217-1235.
41. Mazzone ES, Vasco G, Palermo C et al. A critical review of functional assessment tools for upper limbs in Duchenne muscular dystrophy. *Dev Med Child Neurol* 2012;54:879-885.
42. Mercuri E. McDonald C, Mayhew A et al. International workshop on assessment of upper limb function in Duchenne muscular dystrophy: Rome, 15-16 February 2012. Neuromuscul Disord 2012;22:1025-1028.
43. Berard C, Payan C, Hodgkinson I, Fermanian J. A motor function measure for neuromuscular diseases. Construction and validation study. *Neuromuscul Disord* 2005;15:463-470.
44. Lyle RC. A performance test for assessment of upper limb function in physical rehabilitation treatment and research. *Int J Rehabil Res* 1981;4:483-492.
45. Yozbatiran N, Der-Yeghiaian L, Cramer SC. A standardized approach to performing the action research arm test. *Neurorehabil Neural Repair* 2008;22:78-90.
46. Bartels B, Pangalila RF, Bergen MP, Cobben NA, Stam NJ, Roebroeck ME. Upper limb function in adults with Duchenne muscular dystrophy. *J Rehabil Med* 2011;43:770-775.
47. Mayhew A, Mazzone ES, Eagle M et al. Development of the Performance of the Upper Limb module for Duchenne muscular dystrophy. *Dev Med Child Neurol* 2013;55:1038-1045.

48. Scott E, Eagle M, Mayhew A et al. Development of a functional assessment scale for ambulatory boys with Duchenne muscular dystrophy. *Physiother Res Int* 2012;17:101-109.
49. Finanger EL, Russman B, Forbes SC, Rooney WD, Walter GA, Vandenborne K. Use of skeletal muscle MRI in diagnosis and monitoring disease progression in Duchenne muscular dystrophy. *Phys Med Rehabil Clin N Am* 2012;23:1-10,ix.
50. Hollingsworth KG, Garrood P, Eagle M, Bushby K, Straub V. Magnetic resonance imaging in duchenne muscular dystrophy: longitudinal assessment of natural history over 18 months. *Muscle Nerve* 2013;48:586-588.
51. McDonald CM, Henricson EK, Abresch RT et al. The 6-minute walk test and other clinical endpoints in Duchenne muscular dystrophy: Reliability, concurrent validity, and minimal clinically important differences from a multicenter study. *Muscle Nerve* 2013;48:357-368.
52. Henricson ER, Abresch JJ Han et al. The 6-minute walk test and person-reported outcomes in boys with duchenne muscular dystrophy and typically developing controls: longitudinal comparisons and clinically-meaningful changes over one year. *PLoS Curr* 2013;5.
53. Arpinelli F, Bamfi F. The FDA guidance for industry on PROs: the point of view of a pharmaceutical company. *Health Qual Life Outcomes* 2006;4:85.
54. Speight J,SM Barendse. FDA guidance on patient reported outcomes. *BMJ* 2010;340:c2921.
55. Bendixen RM, Senesac C, Lott DJ, Vandenborne K. Participation and quality of life in children with Duchenne muscular dystrophy using the International Classification of Functioning, Disability, and Health. *Health Qual Life Outcomes* 2012;10:43.
56. Baiardini I, Minetti C, Bonifacino S et al. Quality of life in Duchenne muscular dystrophy: the subjective impact on children and parents. *J Child Neurol* 2011;26:707-713.
57. Davis SE, Hynan LS, Limbers CA et al. The PedsQL in pediatric patients with Duchenne muscular dystrophy: feasibility, reliability, and validity of the Pediatric Quality of Life Inventory Neuromuscular Module and Generic Core Scales. *J Clin Neuromuscul Dis* 2010;11:97-109.
58. Allen DG. Eccentric muscle damage: mechanisms of early reduction of force. *Acta Physiol Scand* 2001;171:311-319.
59. Petrof BJ. The molecular basis of activity-induced muscle injury in Duchenne muscular dystrophy. *Mol Cell Biochem* 1998;179:111-123.
60. Sandri M, Carraro U, Podhorska-Okolov M et al. Apoptosis, DNA damage and ubiquitin expression in normal and mdx muscle fibers after exercise. *FEBS Lett* 1995;373:291-295.
61. Lim JH, Kim DY, Bang MS. Effects of exercise and steroid on skeletal muscle apoptosis in the mdx mouse. *Muscle Nerve* 2004;30:456-462.
62. Vilquin JT, Brussee V, Asselin I, Kinoshita I, Gingras M, Tremblay JP. Evidence of mdx mouse skeletal muscle fragility in vivo by eccentric running exercise. *Muscle Nerve* 1998;21:567-576.
63. Markert CD, Case LE, Carter GT, Furlong PA, Grange RW. Exercise and Duchenne muscular dystrophy: where we have been and where we need to go. *Muscle Nerve* 2012;45:746-751.
64. Jeannet PY, Aminian K, Bloetzer C, Najafi B, Paraschiv-Ionescu A. Continuous monitoring and quantification of multiple parameters of daily physical activity in ambulatory Duchenne muscular dystrophy patients. *Eur J Paediatr Neurol* 2011;15:40-47.
65. Humbertclaude V, Hamroun D, BezzouK et al. Motor and respiratory heterogeneity in Duchenne patients: implication for clinical trials. *Eur J Paediatr Neurol* 2012;16:149-160.
66. van den Bergen JC, Wokke BH, Janson AA et al. Dystrophin levels and clinical severity in Becker muscular dystrophy patients. J Neurol Neurosurg Psychiatry, Published online first 29 Nov 2013. *Published online first: 17 Oct 2013*

Appendix

What can we learn from assisted bicycle training in a girl with Duchenne muscular dystrophy? A case study

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Abstract

In this case study a 9-year old ambulatory girl with Duchenne muscular dystrophy (DMD) due to a mosaic translocation mutation participated in dynamic training. Since the role of exercise is unclear in both boys and girls with DMD, a recently developed assisted bicycle training regimen was evaluated for its feasibility and effectiveness in this girl. The girl trained at home, first 15 minutes with her legs and then 15 minutes with her arms, 5 times a week, for 24 weeks. This case study showed that the training was feasible and safe. Additionally, we found that no physical deterioration occurred during the training period: the Motor Function Measure and the Assisted Six-Minute Cycling Test results remained stable. Slight improvements in quantitative muscle ultrasound intensity were found, indicating less fatty infiltration in the muscles. These results suggest that physical training could beneficial in females with DMD who express low levels of dystrophin.

Key words: Duchenne muscular dystrophy, female, Becker muscular dystrophy, exercise, assisted bicycle training

Introduction

A deficit of the protein dystrophin causes dystrophinopathies like Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD). Absence or near-absence (<3%) of dystrophin results in progressive muscle wasting and eventually loss of function. Dystrophin is encoded by the *DMD* gene located on the short arm of the X-chromosome. The pattern of inheritance of dystrophinopathies is X-linked recessive and, therefore, primarily boys are affected. Female carriers who inherit mutations in the *DMD* gene are generally asymptomatic[1], because they usually express sufficient amounts of dystrophin (~50%) from one X-chromosome to prevent symptoms.[2] However, some girls develop clinical signs similar to DMD, because of several genetic mechanisms that reduce residual dystrophin expression (e.g. skewed X-inactivation[3], chromosomal rearrangements[4] or additional syndromes like Turner's syndrome[5]).

There is no curative treatment for DMD, but improved health care has increased life expectancy in the Western world.[6] Muscle training regimens associated with high levels of mechanical stress may be harmful in the setting of dystophin deficiency, whereas low-stress exercise may produce beneficial effects on myofiber contractility and energetic defiency.[7] It is hypothesized that non-strenuous exercise can potentially be used to avoid disuse atrophy and other secondary complications of physical inactivity.[8] Health care guidelines, therefore, recommend participation in regular sub-maximum functional activities and avoidance of exhausting high-resistance strength and eccentric training,[8] but this is based on limited evidence. Only five randomized controlled trials (RCT's) have been performed in adults with muscle diseases, from which no general training advices can be given.[9] Only one case report on an adult symptomatic DMD carrier exists showing that resistance exercise results in increased muscle strength and a decreased number of falls.[10]

In this case report, we describe a girl with DMD who received assisted bicycle training for her legs and arms. A recently finished RCT concluded that this training maintained the functional abilities in ambulant and wheelchair-dependent boys with DMD during 24 weeks of training (manuscript accepted for publication in Neurorehabilitation and Neural Repair). We assessed whether this training was also safe and beneficial in this girl with DMD. The results of this case report provide some insight into the influence of a certain level of dystrophin on the effects of exercise.

Case Summary

A 9-year old ambulatory girl with DMD participated in assisted bicycle training. The girl had muscle weakness, and used a buggy for long distances. She participated in yoga and swimming and had hydrotherapy, all for one hour per week.

The first signs of delayed motor skill development were noticed by her parents at the age of 11 months as she did not shift position at night and could not move her head from left to right. A muscle disease was suspected due to her enlarged calves and Gower's sign. At the age of 2.5 years, her creatine kinase level was above 28.000 U/L (normal < 200 U/L). At the age of 4 years the diagnosis DMD was established by a *de novo* translocation between the short arm of the X-chromosome (in frame the DMD gene) and the long arm of chromosome 13 [t(X; 13)(p21;q22)] and a selective inactivation of the normal X-chromosome A muscle biopsy of the quadriceps muscle exhibited 26.3% (76 out of 267 fibers) dystrophin-positive fibers distributed in a mosaic pattern. She received the same rehabilitative care as DMD boys and was put on an oral intermittent (ten days on, ten days off) prednisolone regimen (dose 0.75 mg/kg) from the age of 7 years onwards, which was continued during the training period.

Assisted bicycle training

The girl performed training as a part of the No Use is Disuse (NUD) study, which was approved by our regional Medical Ethics Committee. Parents provided informed consent. An extensive description of the 24 weeks assisted bicycle training protocol is freely available online.[11] The girl trained at home, 15 minutes with her legs followed by her arms, 5 times a week at an assistance level passive mode 1 (i.e. no-load, speed 7 revolutions per minute). The training intensity was low-to-moderate as established with the OMNI scale of perceived exertion (max OMNI score=6).

Outcome parameters

Figure 1 shows the clinical assessments (T0-T6) throughout the training period. Assessments were performed at the Department of Rehabilitation (T0,T2,T4,T6) and at home (other assessments). The primary endpoint was after 24 weeks of training (T4). The primary outcomes were the Motor Function Measure (MFM) and the Assisted Six-Minute Cycling Test (A6MCT). Secondary outcomes were the Vignos and Brooke scale for lower and upper extremity functioning, timed tests (time to rise from a floor, to rise from a chair, to climb 3 stairs and to walk 10 meters), the Medical Research Scale (MRC) for muscle strength, and quantitative muscle ultrasound (QMUS) to determine the echo intensity (EI) of the biceps brachii, the forearm flexors, the rectus femoris and the tibialis anterior. EIs are presented as z-scores, i.e. the number of SD from the mean of the reference group. In boys with DMD, EIs increase with age and as the disease severity worsens due to muscle fibrosis.

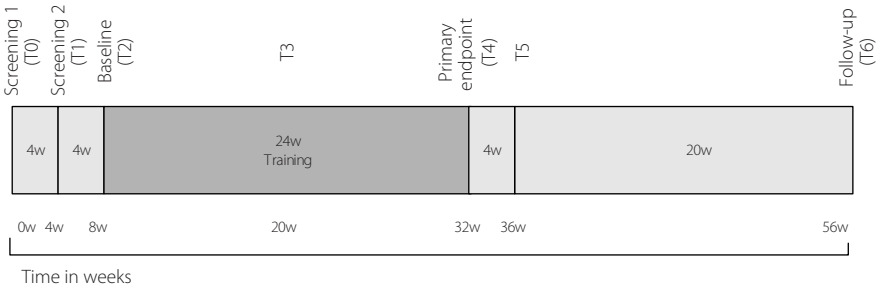

Figure 1 Clinical assessments throughout the study.

Dark grey area: training period; T2: one day before the start of the training period; T4: one day after the end of the training period.

Performance and disease progression

The girl completed the entire training program while no adverse effects were recorded. During each training session she achieved on average 1068 (SD±43.3) revolutions with the legs and 992 revolutions (SD±82.8) with the arms. Her median OMNI score was 1 (range 0-3) for the legs, 3.5 (range 2-9) for the arms, and 0.5 (range 0-6) globally.

Table 1 shows the study outcomes. With respect to the primary study outcomes, the MFM remained stable over time (mean=84.1, SD±1.3, range 82-86), although a small drop was observed at T3 due to a decrease in the score of dimension 1 (standing and transfers). The A6MCT showed no major changes in the number of revolutions achieved with the legs (mean=576, SD±28) or arms (mean=501, SD±49) (revolution reference values for aged matched DMD boys: legs mean=405, SD±152 and arms mean=370, SD±120; revolution reference values for healthy aged matched boys: legs mean=843, SD±82 and arms mean=778, SD±111).[12]

None of the secondary outcomes showed a regression either during training or follow-up, except the time it took to rise from the floor. During the latter task, the girl showed a wide variability in both quality (range 3-5, i.e. without hand-support to bilateral hand-support) and time (range 3.5-4.9 seconds), particularly at T3. At start of the training (T2), the ultrasound muscle EI was high in all four muscles (EI > 47, *z*-scores ranged from 2.3 to 5.9) indicating muscle fibrosis and fatty infiltration. After 24 weeks of training (T4) all EIs decreased with 0.1 to 1.1 *z*-scores (*z*-scores ranged from 1.2 to 3.7), with the exception of the rectus femoris (*z*-score at T2=5.9, and at T4=6.1). Only the rectus femoris and tibialis anterior showed abnormal EIs after 24 weeks of training (EI > 62, *z*-scores >2).

Table 1 Training results on the primary and secondary outcomes

	T_N: measurement						
	T_0	T_1	T_2	T_3	T_4	T_5	T_6
General data							
Measurement moment (in weeks)	0	4	8	20	32	36	56
Corticosteroids Number of days on/off	On 10	Off 5	Off 2	Off 4	On 8	On 10	Off 6
Primary outcomes							
MFM score (total 96) (%)	84 (87.5)	84 (87.5)	85 (88.5)	82 (85.4)	85 (88.5)	86 (89.6)	83 (86.4)
MFM_D1 (total 39) (%)	30 (76.9)	29 (74.4)	29 (74.4)	25 (64.1)	28 (71.8)	30 (76.9)	26 (66.7)
MFM_D2 (total 36) (%)	35 (97.2)	35 (97.2)	36 (100)	36 (100)	36 (100)	36 (100)	36 (100)
MFM_D3 (total 21) (%)	19 (90.4)	20 (95.2)	20 (95.2)	21 (100)	21 (100)	20 (100)	21 (100)
A6MCT for the arms (Revolutions)	529	-	442	-	552	-	482
A6MCT for the legs (Revolutions)	606	-	594	-	547	-	557
Secondary outcomes							
Vignos (range 1-10)	2	2	2	2	2	2	2
Brooke (range 1-6)	1	1	1	1	1	1	1
Timed tests							
Gait Quality (range 1-7) Time (seconds)	 3 4.9	 - -	 3 5.3	 - -	 3 5.3	 - -	 3 5.8
Stairs Quality (range 1-7) Time (seconds)	 1 2.0	 - -	 1 3.6	 - -	 1 2.6	 - -	 1 2.7
Gowers Quality (range 1-7) Time (seconds)	 4 4.6	 5 4.8	 3 3.5	 5 4.9	 3 4.1	 5 3.6	 5 4.3

Table 1 Continued

	T_N: measurement						
	T_0	T_1	T_2	T_3	T_4	T_5	T_6
Secondary outcomes							
Chair Quality (range 1-6) Time (seconds)	 1 0.6	 - -	 2 1.0	 2 1.5	 2 1.6	 - -	 2 1.6
Nine-hole Peg Test (seconds)	19.1	20.0	19.4	17.2	17.5	17.8	16.4
MRC scale (range 0-5)							
Gluteus left	2	2	3	3	3	3	3
Gluteus right	2	3	3	3	3	3	3
Quadriceps left	5	4	5	5	5	5	5
Quadriceps right	5	4	5	5	5	5	4
Tibialis anterior left	4	4	4	4	5	5	5
Tibialis anterior right	4	4	4	4	5	5	4
Deltoid left	4	4	4	4	4	4	4
Deltoid right	4	4	4	4	4	4	4
Triceps left	4	4	4	4	4	4	4
Triceps right	4	4	4	4	4	4	4
QMUS (mean EI/ *z*-score)							
Biceps brachii, left			47.8/ 2.3		39.2/ 1.2		
Forearmflexors, right			48.1/ 2.5		45.4/ 2.0		
Rectus femoris, right Tibialis anterior, left			64.7/ 5.9 64.1/ 3.8		66.9/ 6.1 62.9/ 3.7		

T0 to T2 represent the multiple baseline measurements. The girl started training at T2 and continued training until T4. T4 and T5 represent follow-up assessments.
Abbreviations: MFM: Motor Function Measure; D: domain; A6MCT: Assisted Six-Minute Cycling Test; Gait: walk 10 meters; Stairs: climb three stairs; Gowers: rise from the floor; Chair: rise from a chair; MRC scale: Medical Research Council scale; QMUS: Quantitative muscle ultrasound; EI: echo intensity

Discussion

The girl with DMD described in this case study showed an overall stable performance in outcome parameters, indicating a stability of the functional abilities and endurance during 24 weeks of assisted bicycle training. Only the MFM and the time to rise from the floor showed a temporary regression after 12 weeks of training which can be explained by either the concurrent corticosteroid free period in contrast to baseline, or natural

fluctuations. Notably, the current case study indicates that dynamic training is feasible and safe in girls with DMD. This is in accordance with the results of 30 boys with DMD who participated in the same assisted bicycle training: the training was feasible and safe for both ambulatory and wheelchair-dependent boys, and the training delayed the functional deterioration.[13]

The stable functional abilities in the girl described in this case study could also be explained by a BMD-like instead of a DMD-like disease progression. We found 26% dystrophin positive fibers in the quadriceps muscle, because not all her cells contained the translocation -. Of course with a mosaic pattern it is possible that dystrophin levels in other muscles are different, which may explain why this girl presents like a DMD instead of a BMD. A longer baseline, and follow-up period with multiple assessments would have provide information about the natural progression of her disease.

It remains to be elucidated whether improvements in functional abilities and endurance could be achieved with a higher training intensity, a low resistance training or a longer training period (i.e. more than 24 weeks). Nevertheless, the number of revolutions and the achieved scores on the OMNI scale indicated that this training intensity was preferred. Only for the legs the OMNI score was less than 3, so the training intensity could probably be higher.

Next to the stable functional abilities, the girl showed a slight improvement in muscle composition at the end of the training period, i.e. she had less fatty infiltration compared to baseline. This is in accordance with findings from *mdx* mice which also showed training-induced improvements in pathology.[14] In addition, a study in a mouse model expressing varying low dystrophin levels showed that mice expressing >20% dystrophin are protected against exercise-induced damage.[15] In our case, the girl had a dystrophin level of >20%, and might be protected from exercise-induced damage.

This case report suggests that assisted bicycle training is a suitable training for patients with DMD because it is feasible and safe. Moreover, assisted bicycle training might potentially improve muscle function, because the girl in this case report showed a stable motor function as well as an improvement in muscle composition during the intervention period. In addition, the relation between dystrophin expression and exercise may be well studied in girls with DMD expressing low levels of dystrophin.

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Author contributions

JH wrote the first draft of the manuscript and prepared the figure and table. MJ wrote the final study protocol (revised by NvA and IdG), recruited the participant, performed the clinical assessments, coached the participant during her training, and modified subsequent drafts of the manuscript. IG, ML and MvP critically reviewed the manuscript. ML also investigated the translocation of the participant and provided genetic information. NvA and IdG handled funding, developed the first draft of the protocol and critically reviewed the manuscript. Moreover, IdG acted as the study supervisor.

Declaration of conflicts of interests

The authors declared no potential conflicts of interests with respect to the research, authorship, and/ or publication of this manuscript.

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Ethical approval

The girl performed the training program as a part of the No Use is Disuse (NUD) study, which was approved by our regional Medical Ethics Committee.

References

1. Hoogerwaard EM, Bakker A, Ippel PF et al. Signs and symptoms of Duchenne muscular dystrophy and Becker muscular dystrophy among carriers in The Netherlands: a cohort study. *Lancet*. 1999; 353(9170): 2116-2119.
2. Seemann N, Selby K, McAdam L, et al. Symptomatic dystrophinopathies in female children. *Neuromuscul Disord*. 2011; 21(3): 172-177.
3. Yoshioka M, Yorifuji T, Mituyoshi I. Skewed X inactivation in manifesting carriers of Duchenne muscular dystrophy. *Clinical genetics*; 1998; 53(2): 102-107.
4. Verellen-Dumoulin C, Freund M, De Meyer R, et al. Expression of an X-linked muscular dystrophy in a female due to translocation involving Xp21 and non-random inactivation of the normal X chromosome. *Human genetics*. 1984; 67(1): 115-119.
5. Chelly J, Marlhens F, Le Marec B, et al. De novo DNA microdeletion in a girl with Turner syndrome and Duchenne muscular dystrophy. *Human genetics*. 1986; 74(2): 193-196.
6. Kieny P, Chollet S, Delalande P, et al. Evolution of life expectancy of patients with Duchenne muscular dystrophy at AFM Yolaine de Kepper centre between 1981 and 2011. *Annals of physical and rehabilitation medicine*. 2013; 56(6): 443-454.
7. Petrof BJ. The molecular basis of activity-induced muscle injury in Duchenne muscular dystrophy. *Molecular and cellular biochemistry*. 1998; 179(1-2): 111-123.
8. Bushby K, Finkel R, Birnkrant DJ, et al. Diagnosis and management of Duchenne muscular dystrophy, part 2: implementation of multidisciplinary care. *Lancet Neurol*. 2010; 9(2): 177-189.
9. Voet NB, van der Kooij EL, Riphagen II, et al. Strength training and aerobic exercise training for muscle disease. *Cochrane Database Syst Rev*. 2013:CD003907.
10. Bohannon RW, Jones PL. Results of manual resistance exercise on a manifesting carrier of Duchenne muscular dystrophy. A case report. *Phys Ther*. 1986; 66(6): 973-975.
11. Jansen M, de Groot IJM, van Alfen N, Geurts ACH. Physical training in boys with Duchenne Muscular Dystrophy: the protocol of the No Use is Disuse study. *BMC Pediatr*. 2010; 10: 55.
12. Jansen M, De Jong M, Coes HM, et al. The assisted 6-minute cycling test to assess endurance in children with a neuromuscular disorder. *Muscle Nerve*. 2012; 46(4): 520-530.
13. Jansen M, Van Alfen N, Geurts ACH, De Groot IJM. Assisted bicycle training delays functional deterioration in boys with Duchenne muscular dystrophy: the randomized controlled trial "No Use is Disuse". *Neurorehabil Neural Repail*. 2013; 27(9): 816-827.
14. Hayes A, Williams DA. Beneficial effects of voluntary wheel running on the properties of dystrophic mouse muscle. *J Appl Physiol*. 1996; 80(2): 670-679.
15. van Putten M, Hulsker M, Nadarajah VD, et al., The effects of low levels of dystrophin on Mouse muscle function and pathology. *PLoS One*. 2012; 7(2): e31937.

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Curriculum Vitae

Samenvatting

Duchenne spierdystrofie (DMD) is een is een erfelijke spierziekte die voorkomt bij gemiddeld 1/5000 jongens. De afwezigheid van dystrofine zorgt voor spierafbraak en verlies van spierkracht en functionele mogelijkheden. Jongens met DMD raken rond de leeftijd van 10 jaar rolstoelafhankelijk en kunnen daarna al snel de armen niet meer optillen om bijvoorbeeld te eten. Aangezien er op dit moment nog geen genezing mogelijk is, is een belangrijk behandeldoel het vertragen van de functionele achteruitgang. De functionele achteruitgang komt primair door de spierziekte zelf, maar kan worden versneld door 'disuse'. Onder disuse wordt hier verstaan de discrepantie tussen dat wat jongens kunnen (hun capaciteit) en dat wat ze doen (hun activiteit). Met andere woorden, de moeite die het kost om te bewegen leidt tot minder bewegen c.q. lichamelijke inactiviteit die weer leidt tot secundaire lichamelijke achteruitgang.

De huidige internationale richtlijnen raden jongens met DMD lichamelijke activiteiten met een milde intensiteit aan om de secundaire lichamelijke achteruitgang tegen te gaan. Deze richtlijnen zijn echter weinig specifiek en zijn gebaseerd op een beperkt aantal onderzoeken. De gangbare trainingsprincipes maken doorgaans gebruik van weerstand en kunnen te zwaar zijn voor jongens met DMD. Uitputting van de spieren tijdens training wordt afgeraden, omdat dit de spierafbraak kan versnellen bij DMD. Een training waarbij ondersteuning wordt geboden, bijvoorbeeld in de vorm van een fiets met traponder-steuning, kan hiervoor uitkomst bieden. Het doel van de onderzoeken die worden beschreven in dit proefschrift was het ontwikkelen van een haalbare training met mechanische ondersteuning en het evalueren van de effectiviteit hiervan bij jongens in diverse stadia van DMD.

Introductie

Hoofdstuk 1 is de algemene introductie en beschrijft kort de pathofysiologie van DMD, het beloop van de ziekte, en de behandeling (inclusief de fysiotherapie). Het beschrijft het 'disuse' fenomeen, de rol van training, en de noodzaak voor nieuwe gevalideerde uitkomstmaten die het beloop van de spierziekte kunnen meten. Vervolgens worden het doel van het proefschrift en de twee belangrijkste vraagstellingen geformuleerd:

1) Zijn kwantitatieve spierechografie, de zes-minuten fietstest met mechanische ondersteuning en de KIDSCREEN-52 vragenlijst valide en sensitieve meetinstrumenten om de effectiviteit van fysieke training bij jongens met DMD te evalueren?
2) Is een training met mechanische ondersteuning haalbaar, veilig en effectief om de functionele achteruitgang te vertragen bij ambulante (lopende) en rolstoelafhankelijke jongens met DMD?

De introductie eindigt met een beschrijving van de opbouw van dit proefschrift. De kern van dit proefschrift bestaat uit twee delen. Deel 1 beschrijft de ontwikkeling en validatie van drie *meetinstrumenten* die zijn gebruikt om de effectiviteit van fysieke training met mechanische ondersteuning te evalueren, namelijk: kwantitatieve echografie van spieren (spierechografie) om spierveranderingen te meten (**hoofdstuk 2**), de zes-minuten fietstest met mechanische ondersteuning om het uithoudingsvermogen te meten (**hoofdstuk 3**), en de KIDSCREEN-52 vragenlijst om de gezondheidsgerelateerde kwaliteit van leven te meten (**hoofdstuk 4**). In deel 2 worden het protocol (**hoofdstuk 5**) en de resultaten (**hoofdstuk 6 en 7**) van twee trainingsprogramma's beschreven die samen de "No Use is Disuse (NUD) study" vormen. De bevindingen worden samengevat en bediscussieerd in **hoofdstuk 8**.

Deel 1 Meetinstrumenten voor Duchenne spierdystrofie

Het doel van **hoofdstuk 2** was het onderzoeken of kwantitatieve spierechografie een geschikt meetinstrument is voor interventiestudies bij DMD. Met kwantitatieve spierechografie kunnen de echo-intensiteit, ofwel de grijswaarde, en spierdikte worden gemeten. De verlittekening en vervetting van de spieren is bij jongens met DMD te zien als een verhoogde echo-intensiteit. Dit betekent dat de spieren van jongens met DMD met spierechografie witter zijn dan van gezonde jongens. In dit onderzoek werd gekeken of het met kwantitatieve spierechografie mogelijk is veranderingen te meten naarmate jongens ouder worden. Er is ook gekeken of deze veranderingen gerelateerd zijn aan veranderingen in ziekte-ernst zoals gemeten met verschillende fysiotherapeutische tests (de Vignos en Brook schaal, spierkracht, de Childhood Myositis Assessment Scale, en de Hammersmith Motor Ability Scale). Achttien jongens in de leeftijd van 3.7 tot 15.1 jaar (15 lopers, 3 rolstoelafhankelijk) kregen elk twee tot vijf keer een spierecho gedurende een mediane periode van 27.5 maanden. De jongens kregen een spierecho van twee beenspieren (de m. rectus femoris en m. tibialis anterior) en twee armspieren (de m. biceps brachii en de flexoren van de onderarm). Elf van de achttien jongens werden ook getest door de fysiotherapeut. Zoals verwacht lieten de resultaten zien dat de echo-intensiteit toenam naarmate jongens ouder werden en lichamelijk achteruit gingen. De echo-intensiteit van de beenspieren was het meest gevoelig om veranderingen na een jaar aan te tonen. De spierdikte liet geen afname zien naarmate jongens ouder werden. De resultaten van dit onderzoek laten zien dat kwantitatieve spierechografie een praktisch, kindvriendelijk en betrouwbaar meetinstrument is voor de follow-up van jongens met DMD.

Hoofdstuk 3 beschrijft de ontwikkeling en validatie van een nieuwe submaximale conditietest: de zes-minuten fietstest met mechanische ondersteuning. Submaximale conditietesten zijn de primaire uitkomstmaat in lopende klinische studies bij kinderen met een neuromusculaire aandoening (NMA), omdat zij gerelateerd zijn aan het functioneren in het dagelijks leven. Op dit moment is de meest gebruikte submaximale

conditietest voor kinderen met een NMA de zes-minuten looptest. Deze test is echter niet geschikt voor jongens met DMD die aan het einde van hun loopfase zijn of rolstoelafhankelijk. Aangezien er momenteel interventiestudies worden opgezet voor oudere jongens met DMD is een geschikte submaximale conditietest nodig voor deze groep jongens. Het doel van dit onderzoek was een dergelijke test te ontwikkelen. Dit is de zes-minuten fietstest met mechanische ondersteuning geworden. Tijdens deze test proberen jongens in zes minuten tijd zoveel mogelijk omwentelingen te behalen met de benen en/of de armen op een fietsje met mechanische ondersteuning. De ondersteuning betekent dat als de jongens niet fietsen de trappers toch zeven keer per minuut rondgaan. Zo is het ook voor jongens met weinig spierkracht mogelijk te fietsen. In het tweede deel van het onderzoek is de uitvoerbaarheid van de zes-minuten fietstest beoordeeld bij gezonde jongens (n= 99) en jongens met DMD (n=30). Bij de gezonde jongens zijn ook de test-hertest betrouwbaarheid en de relatie met de zes-minuten looptest onderzocht. Bij de jongens met DMD is de relatie tussen de zes-minuten fietstest en de ziekte-ernst zoals gemeten met de Motor Function Measure (MFM) onderzocht. De resultaten lieten zien dat de fietstest voor de benen uitvoerbaar was voor 99% van de gezonde jongens en 97% van de jongens met DMD. De fietstest voor de armen was uitvoerbaar voor alle gezonde jongens en jongens met DMD. Zoals verwacht behaalden jongens met DMD minder omwentelingen met de benen en armen dan gezonde jongens. Bij de gezonde jongens was de fietstest reproduceerbaar. Zowel de test voor de benen ($r = 0.58$, $p < 0.01$) als de armen ($r = 0.65$, $p < 0.01$) correleerden positief met de zes-minuten looptest. In de totale groep van jongens met DMD correleerde de fietstest voor de benen positief met de totaalscore van de MFM ($\rho = 0.65$, $p < 0.01$). Bij rolstoelafhankelijke jongens met DMD correleerde ook de fietstest voor de armen positief met de MFM ($\rho = 0.84$, $p < 0.01$). De zes-minuten fietstest is derhalve een geschikt meetinstrument voor longitudinale studies bij kinderen met een NMA die de mogelijkheid om te lopen verliezen.

Hoofdstuk 4 beschrijft de gezondheidsgerelateerde kwaliteit van leven bij jongens met DMD. De gezondheidsgerelateerde kwaliteit van leven wordt gezien als een belangrijke uitkomstmaat in klinisch onderzoek, omdat het inzicht geeft in de ervaren gezondheid van de deelnemers. Dit onderzoek had drie doelen. Als eerste zijn verschillende domeinen van kwaliteit van leven (zoals lichamelijk welzijn en zelfbeeld) onderzocht bij veertig jongens met DMD. Negentien van deze veertig jongens waren lopers, 14 jongens waren rolstoelafhankelijk en hadden een goede armfunctie en 7 jongens waren rolstoelafhankelijk en hadden een beperkte armfunctie. Als tweede is de gezondheidsgerelateerde kwaliteit van leven van deze jongens vergeleken met de perceptie van de ouders. Als laatste is de relatie tussen de gezondheidsgerelateerde kwaliteit van leven en de functies en activiteiten van de jongens onderzocht. De verwachting was dat DMD een negatieve invloed zou hebben op de gezondheidsgerelateerde kwaliteit van leven, wat gunstig beïnvloed zou kunnen worden door een interventie. De gezondheidsgerelateerde

kwaliteit van leven is gemeten met de KIDSCREEN-52 vragenlijst. De scores van de jongens met DMD zijn vergeleken met normwaarden van gezonde Nederlandse leeftijdsgenoten. De resultaten lieten zien dat jongens met DMD alleen hun "Lichamelijk welzijn" minder goed ervaren dan gezonde leeftijdsgenoten. De gezondheidsgerelateerde kwaliteit van leven was niet gerelateerd aan de functies en activiteiten van de jongens. Dit betekent dat DMD en de progressie van de ziekte de gezondheidsgerelateerde kwaliteit van leven niet nadelig beïnvloeden. De perceptie van de ouders is echter dat hun zoons lager scoren op de domeinen "Zelfbeeld", "Stemming en emoties", en "Pesten". Het is belangrijk ouders te wijzen op deze onderwaardering.

Deel 2 Training bij jongens met Duchenne spierdystrofie

Hoofdstuk 5 beschrijft de onderzoeksvragen, hypothesen en onderzoeksmethoden van de NUD studie. De NUD studie is het eerste gerandomiseerd gecontroleerde onderzoek (RCT) naar de effecten van fysieke training bij jongens met DMD. Het is ook het eerste onderzoek dat de effecten van een fysieke training met mechanische ondersteuning heeft onderzocht. De hypothese was dat regelmatige training met ondersteuning, en dus met een lage trainingsintensiteit, de secundaire functionele achteruitgang als gevolg van disuse zou tegengaan. De NUD studie bestond uit twee sub-studies die de uitvoerbaarheid, veiligheid en effectiviteit van (1) een fietstraining voor de benen en armen met traponder-steuning en (2) een functionele training met dynamische armondersteuning hebben onderzocht.

Substudie 1 (zie hoofdstuk 6) was een RCT met multipele baselinemetingen. Het doel was 30 jongens met DMD mee te laten doen die aan het eind van hun loopfase waren of recent rolstoelafhankelijk waren geworden. Jongens werden op basis van loting toegewezen aan een trainings- of een controlegroep. Na een periode van acht weken waarin uitgangsmetingen werden gedaan startte de trainingsgroep met de 24-weken durende fietstraining, terwijl de controlegroep geen specifieke interventie kreeg (standaard zorg). De trainingsgroep fietste vijf keer per week, 15 minuten met de benen en 15 minuten met de armen. Het fietsen deden zij op een fiets (bewegingstrainer) met ondersteuning, die zorgde voor ten minste zeven omwentelingen per minuut als er niet actief gefietst werd. Jongens werden geïnstrueerd met een constant tempo van ongeveer 65 omwentelingen per minuut te fietsen met een ervaren vermoeidheid van "een beetje moe" tot "iets meer moe". De ervaren vermoeidheid werd gemeten met de OMNI schaal voor ervaren vermoeidheid. Jongens trainden thuis of op school. De controlegroep kreeg dezelfde fietstraining na een controleperiode (wachtlijstperiode). De trainings- en controlegroep zijn gemeten tot respectievelijk 56 en 60 weken na de start van de studie. De primaire uitkomstmaten waren de MFM en de zes-minuten fietstest met ondersteuning voor de benen en armen. De MFM meet de motorische functies (in deze studie omschreven als de functionele mogelijkheden), terwijl de zes-minuten fietstest met ondersteuning het submaximale uithoudingsvermogen meet. Secundaire uitkomstmaten

waren de Pediatric Evaluation of Disability Inventory (PEDI), tijdsgebonden functionele testen, spierkracht, gewrichtsmobiliteit en spierechografie. Bijwerkingen tijdens de trainingsperiode zijn gemeten met vragenlijsten en huisbezoeken door de onderzoeker. Groepsverschillen tussen de trainings- en controlegroep zijn gemeten met een covariantieanalyse (ANCOVA). Het primaire eindpunt was na 24 weken trainen/controle periode.

Substudie 2 (zie hoofdstuk 7) was haalbaarheidsstudie zonder controle personen. Het doel was tien rolstoelafhankelijke jongens met DMD mee te laten doen die problemen hadden met het reiken en optillen van de armen. Na een baselineperiode van acht weken kregen alle jongens een dynamische armondersteuner voor hun niet-dominante arm. Met de armondersteuner trainden zij gedurende 24 weken het voorwaarts en zijwaarts reiken door het spelen van een virtual reality computerspel (vijf dagen per week, vijf spellen per dag). Hierbij werd de jongens gevraagd ten minste twee keer per week met de armondersteuner te eten. De jongens werden na 12 en 24 (primaire eindpunt) weken trainen beoordeeld. Een laatste follow-up meting werd 12 weken na het eind van de training gedaan. Therapietrouw en veiligheid werden gemeten met een tweewekelijkse vragenlijst en huisbezoeken door de onderzoeker. De primaire uitkomstmaat voor het evalueren van de effectiviteit van de training was de Action Research Arm Test (ARAT). De ARAT meet de motorische functies (in deze studie omschreven als de functionele mogelijkheden) van de bovenste extremiteit. Secundaire uitkomstmaten waren de derde dimensie van de MFM ("Distale motorische functies"), de Nine-hole Peg Test, en de Jebsen Taylor Hand Function Test. De haalbaarheid, veiligheid en effectiviteit van de training zijn beschreven per deelnemer. De ongetrainde arm diende hierbij als referentie voor de getrainde arm.

Hoofdstuk 6 beschrijft de resultaten van de fietstraining voor de benen en armen met mechanische ondersteuning (zie hoofdstuk 5). Dertig jongens met DMD met een gemiddelde leeftijd van 10.5 jaar werden ingeloot in een trainings- (n = 17) of controlegroep (n = 13). Achttien jongens waren ambulant en 12 jongens rolstoelafhankelijk. Op één jongen na hebben alle jongens uit de trainingsgroep de fietstraining afgerond. Na 24 weken trainen was de totale MFM score stabiel gebleven in de trainingsgroep terwijl deze was afgenomen in de controlegroep (verschil tussen de trainings- en controlegroep bij 24 weken gecorrigeerd voor baseline = 4.9, 95% betrouwbaarheidsinterval = 2.2 - 7.6). Er zijn geen effecten gevonden voor de zes-minuten fietstest voor de benen (Δ = 5.6, 95% betrouwbaarheidsinterval = –56.2 - 67.3), de armen (Δ = 13.8, 95% betrouwbaarheidsinterval = –60.7 - 88.3) of de secundaire uitkomstmaten. Na de controleperiode hebben acht van de 13 jongens uit de controlegroep alsnog dezelfde fietstraining afgerond. Na 24 weken trainen lieten zij dezelfde stabilisatie op de MFM zien als de trainingsgroep. Er werden geen nadelige bijwerkingen van de training gevonden. De resultaten van dit onderzoek lieten zien dat een fietstraining met mechanische ondersteuning voor de benen en armen haalbaar en veilig is voor ambulante en rolstoelafhankelijke jongens met DMD. De

training vertraagde de secundaire functionele achteruitgang als gevolgd van 'disuse' bij de deelnemers gedurende de trainingsperiode en lijkt hiermee een zinvolle aanvulling op het behandelprogramma van jongens met DMD.

In **hoofdstuk 7** worden de resultaten van de haalbaarheid, veiligheid en effectiviteit van de functionele training met dynamische armondersteuning beschreven. Acht rolstoelafhankelijk jongens in de leeftijd van 12 tot 20 jaar kregen een armondersteuning voor hun niet-dominante arm. Zes van deze jongens hebben het trainingsprogramma afgerond. De andere twee jongens zijn halverwege de training gestopt met deelname aan het onderzoek om praktische redenen. Er zijn geen bijwerkingen van de training gevonden. Vier van de zes jongens die de training hebben afgerond hadden een grotere afname op de ARAT met hun ongetrainde (verschillen in ARAT scores tussen baseline en 24 weken trainen varieerden tussen de -4 en -21 punten) dan getrainde (verschillen in ARAT scores tussen baseline en 24 weken trainen varieerden tussen de -13 en 6 punten) arm. Vergelijkbare resultaten werden gevonden voor de secundaire uitkomstmaten. De resultaten zijn een eerste aanwijzing dat rolstoelafhankelijke jongens met DMD die een beperkte armfunctie hebben hun armen kunnen trainen met dynamische armondersteuning.

In de algemene discussie (**hoofdstuk 8**) worden mechanismen beschreven die de gevonden vertraging in functionele achteruitgang door training met mechanische ondersteuning kunnen verklaren. Er wordt gesteld dat deze vertraging mogelijk te verklaren is door een optelsom van kleine effecten op het niveau van de spieren en gewrichten, het hart- en vaatstelsel, en het centraal zenuwstelsel. Verder onderzoek naar de effecten van fysieke training (al dan niet met mechanische ondersteuning) bij jongens met DMD is nodig om de onderliggende werkingsmechanismen beter te kunnen begrijpen en specifieke trainingsrichtlijnen voor deze jongens te kunnen opstellen. Dit toekomstige onderzoek zal gebruik moeten maken van betrouwbare meetinstrumenten die aansluiten bij het ziektestadium van de deelnemers en (kleine) veranderingen in de loop van de tijd kunnen meten. Op dit moment kan aan jongens met DMD, hun ouders en behandelend (para)medici worden geadviseerd dat een training met mechanische ondersteuning een zinvolle aanvulling kan zijn op het behandelprogramma om de lichamelijke achteruitgang als gevolg van disuse te vertragen.

List of publications

Papers

Jansen M, van Alfen N, Geurts ACH, de Groot IJM. Assisted bicycle training delays functional deterioration in boys with Duchenne Muscular Dystrophy: results of the Randomized Controlled Trial "No Use is Disuse". *Neurorehabil Neural Repair* 2013;27:816-27

Jansen M, Burgers J, Jannink M, van Alfen N, de Groot IJM. Upper limb training with dynamic arm support in boys with Duchenne muscular dystrophy: a feasibility study. *Submitted*

Huijben J, Jansen M, Ginjaar I, Lammens M, van Putten M, van Alfen N, de Groot IJM. What can we learn from assisted bicycle training in a girl with Duchenne muscular dystrophy? A case study. *Submitted*

Opstal SL, Jansen M, van Alfen N, de Groot IJM. Health related quality of life and its relation to disease severity in boys with Duchenne Muscular Dystrophy: satisfied boys, worrying parents. A case control study. *J Child Neurol*, Published online first: 17 Oct 2013

Koene S, Jansen M, Verhaak CM, de Vrueh RLA, de Groot IJM, Smeitink JAM. Towards the harmonisation of outcome measures in children with mitochondrial disorders. *Dev Med Child Neurol* 2013;55:698-706

Jacobs J, Jansen M, Janssen H, Raijmann W, van Alfen N, Pillen S. Quantitative muscle ultrasound and muscle force in healthy children: a 4-year follow-up. *Muscle Nerve* 2013;47:856-63

Jansen M, de Jong M, Coes HM, Eggermont F, van Alfen N, de Groot IJM. The assisted 6-minute cycling test to assess endurance in children with a neuromuscular disorder. *Muscle Nerve* 2012;46:520-30

Jansen M, van Alfen N, Nijhuis- van der Sanden MWG, van Dijk H, Pillen S, de Groot IJM. Quantitative muscle ultrasound is a promising longitudinal follow-up in Duchenne Muscular Dystrophy. *Neuromuscul Disord* 2012;22:306-17

Jansen M, de Groot IJM, van Alfen N, Geurts ACH. Physical training in boys with Duchenne Muscular Dystrophy: the protocol of the No Use is Disuse study. *BMC Pediatr* 2010;10:55

Book chapters

de Groot IJM, Voet NBM, Jansen M, van den Engel-Hoek L. *Rehabilitation in Muscular Dystrophies: Changing Approach*. In: Muscular Dystrophy. Madhuri Hegde and Arunkanth Ankala, Intech, 2012

Anton Comuth, Merel Jansen, Imelda de Groot. *Hoofdstuk 19 Neuromusculaire aandoeningen bij kinderen: veranderde perspectieven. Recente inzichten en ontwikkelingen in de fysiotherapie.* In: Jaarboek Fysiotherapie Kinesitherapie. Bohn Stafleu van Loghum, Houten, Nederland, 2011

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Lieve Rozan, je leest liever Nijntje dan dit boekje, maar een feestje is altijd leuk. Lieve Allard, gaan we nu een datum prikken?

Curriculum Vitae

Merel Jansen was born in Utrecht, the Netherlands, on April 20th 1983. In 2001, she completed her secondary education (HAVO) at the Revius Lyceum Doorn and she started studying physiotherapy at the Hogeschool Utrecht. After obtaining her Bachelor of Health Science degree in 2005, she followed the Master's program Health Sciences with specialization in Prevention and Public Health at the Vrije Universiteit Amsterdam. Meanwhile she worked as a physiotherapist in a health centre. During the Master's program she did her five-month research internship at the Faculty of Sport and Health Sciences at the University of Jyväskylä in Finland. In this internship she focused on determinants of physical activity in youth, which was part of the cross-national survey Health Behaviour in School-aged Children. After obtaining her Master of Science degree in 2007, she started her PhD project at the Department of Rehabilitation at the Radboud University Nijmegen Medical Centre. In this project, called the No Use is Disuse (NUD study), she investigated the effectiveness of assisted physical training in boys with Duchenne muscular dystrophy (DMD) under supervision of dr. Imelda JM de Groot, dr. Nens van Alfen and Prof. dr. Alexander CH Geurts. The NUD study was funded by the patient organization Duchenne Parent Project. During the first two years of her PhD project she also worked part-time as a physiotherapist at a school for chronically ill children. Furthermore, she performed clinical evaluations for an exon-skipping trial at the Radboud University Medical Centre. Currently, she is continuing her research on physical training in boys with DMD at the Department of Rehabilitation at the Radboud University Nijmegen Medical Centre.